Assessing the impacts of methylmercury on piscivorous wildlife using a wildlife criterion value based on the Common Loon

(BRI 2002-08)

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(Report BRI2002-08)

2001 Final Report

Submitted to:

Maine Department of Environmental Protection
Surface Water Ambient Toxic Monitoring Program
State House Station 17
Augusta, Maine 04333

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Executive Summary:

Anthropogenic inputs of mercury (Hg) into the environment have significantly increased in the past few decades. In conjunction, the current availability of methylmercury (MeHg) in aquatic systems has increased to levels posing risks to human and ecological health. Risk levels vary considerably in response to MeHg availability, which is affected by lake hydrology, biogeochemistry, habitat, topography, and proximity to airborne sources. We selected the Common Loon as the most suitable bioindicator of aquatic Hg toxicity, based on ecological, logistical, and other criteria, including public valuations of natural resources. Opportunistic and probability-based sampling efforts from 1994-2001 indicate New England's breeding loon population is at unacceptable levels of risk to Hg contamination, particularly in Maine. Based on risk categories developed from the literature and *in situ* studies by BioDiversity Research Institute and their collaborators, at least 26% of the breeding loon population in Maine is estimated to be at risk, while at least 19% of the eggs laid are potentially impacted.

Because results from national sampling indicated loons were at most risk from Hg in New England (particularly Maine), we identified several individual- and population-level parameters to better understand the extent of mercury toxicity across Maine. Between 1994-01 we collected 199 abandoned eggs (60 in 2001) as well as blood and feather samples from 303 adult (50 in 2001) and 103 juvenile loons captured in Maine. The Hg concentrations in these samples were used to relate sublethal impacts on behavior, developmental stability, immunosuppression, individual survival, egg development, and overall reproductive success. In the Rangeley Lakes Study Area, a total of 181 loon territories were monitored on 44 lakes during 1998-01. Current monitoring efforts and historical data comprise 674 territory-years measured. Behavioral observations were conducted for over 1,500 hours on 16 lakes with 38 loon territories from 1998 to 2000.

Several reproductive measures significantly declined for loon pairs at high risk to prey MeHg availability, thereby corroborating studies in high-risk sites in Nova Scotia and Wisconsin that show Hg impacts reproductive success. Based on 219 loon territories representing 946 territory-years surveyed we found that pairs above the lowest observed adverse effect level (i.e., >3.0 ppm in the blood) fledged 40% fewer young than pairs below our no observed adverse effect level (i.e., <1.0 ppm in the blood). We also found similar significant patterns of lower productivity for other reproductive measures. We view the implication of long-term declines in these reproductive measures as serious and contend they would not be detected by traditional survey techniques.

Insight into why loons are facing Hg-based population declines can be viewed through our hazard assessment process that is based on a weight-of-evidence approach. Physiological impacts of Hg are measured through two key biomarkers: corticosterone stress hormone levels and flight feather asymmetry. Circulating corticosterone hormone levels are strongly linked with increasing blood Hg levels and are not related to capture and handling stress. Corticosterone hormone levels increase on an average of 14.6% for every one ppm of increase in blood Hg levels (n=239). This indicates that loons with high blood Hg levels have higher rates of chronic stress and may therefore have compromised immune systems. Asymmetry measurements provide insights into developmental stability and potentially reproductive fitness. Three years of flight feather measurements have shown agreement among years that loon breeding populations with greater exposure to Hg have significantly greater asymmetry than populations at low risk (n=227). Greater asymmetry may indicate disruptions from stressors on their embryonic development and current physiological status as well as a potential decline in reproductive fitness.

Many behavioral impacts that appear to be related to the neurotoxic effects of MeHg can rarely be observed in the field. We found adult loons in high risk situations left eggs unattended 14% of the time, compared to 1% in controls. Several cases of direct field observations indicate that adult loons with high MeHg body burdens avoid incubating their eggs and display atypical behaviors such as patrolling in front of, or sitting next to the nest. We documented a significant negative relationship between adult blood Hg and foraging behavior, and a significant positive relationship between adult blood Hg and brooding behavior. Recategorizing our data according to energy demands revealed a significant inverse relationship between blood Hg and time spent in high energy behaviors. Our findings are consistent with other studies linking Hg and lethargy, reduced motivation to hunt prey, and compromised foraging abilities.

Current levels of Hg in Maine's lacustrine ecosystems also appear to be impacting individual survival of adult and juvenile loons. Recaptured adult loons exhibit a significant annual increase of Hg (9% in males, 5.6% in females) that we predict will significantly reduce lifetime individual performance. A model of this impact indicates a decline of 13 to 8 young produced over a loon's lifetime. Further, juveniles from high-risk territories have increasing blood Hg levels of 3% per day during the summer, potentially reaching dangerous levels after the final feather molt at 11 weeks of age.

Characterization of the risk imposed by MeHg bioavailability in aquatic systems to high trophic level obligate piscivores such as the Common Loon indicates negative population level impacts in Maine. Although the impacts of Hg on loons are varied, complex, and not yet fully understood, the combination of high exposure to a significant part of the breeding population and the "bottom-line" impact of reducing overall reproductive success to 40%, is creating an aquatic landscape that is not sustainable for the Common Loon in Maine.

Current models indicate a negative population growth rate. Because of the loon's life history strategy (i.e., long lived, slow maturing, and low fecundity) the annual and continual impacts of this type of stressor causes an erosion of the non-breeding or buffer population that serves as a natural cushion to catastrophic events. Once this buffer population is exhausted, the occupancy of established territories will shrink and it will be more obvious that loon populations are declining. However, the realization of shrinking loon populations at that stage will require drastic and potentially expensive efforts to reverse the decline. Models based on a 25-year, statewide comprehensive monitoring effort in New Hampshire show approximately half of Maine's buffer population has been exhausted. Certain areas in Maine, such as the Allagash area that may be particularly impacted from Hg, may already exhibit exhaustion of the buffer population and a shrinking number of territorial pairs.

Continued refinement of model parameters and either a probability-based sampling scheme or new sampling efforts in northern Maine will provide higher confidence in our estimates that will therefore assist in state-based policy efforts as well as national regulations that reflect the ecological injury Hg is currently having on the freshwater landscape.

Our approach to a high resolution risk characterization for the Common Loon provides the necessary information for developing a Maine-based wildlife criterion value (WCV). Recent efforts by the USEPA have established a generic WCV with several major limitations that we are improving with this study. A WCV estimates wildlife population viability through measurement of contaminant stressors such as surface water Hg concentrations.

First-year measurements of exposure parameters indicate a bioaccumulation factor (BAF) of 75,000 for trophic level 3 and 120,000 for trophic level 4 based on the relationship of total Hg in unfiltered water with total Hg in yellow perch. We are not able to calculate a Maine-based reference dose because of several outstanding uncertainties. Further work will correct this limitation and a Maine-based WCV that is protective of aquatic piscivorous wildlife will be obtainable.

Introduction

Due to high concentrations of mercury (Hg) in fish from Maine lakes, ponds, rivers, and streams, the Maine Bureau of Health issued a statewide “fish consumption advisory” in 1994, (modified in 1997) warning Maine citizens to limit consumption of fish from all fresh waters. Impacts on wildlife, however, are less well known in Maine and elsewhere. Recent ecological concerns were highlighted at the “New England Governors and Eastern Canadian Premiers” conference sponsored by the United States Environmental Protection Agency (USEPA) and Maine Department of Environmental Protection (MDEP). Recommendations from the report ‘Northeast States and Eastern Canadian Provinces Mercury Study: A Framework for Action’ stated: “conduct additional research on the cycling and bioavailability of mercury in aquatic ecosystems and on the ecological impacts of elevated fish mercury levels, particularly for fish-eating wildlife such as eagles, loons, osprey, otter, and mink” (NESCAUM 1998).

In addition, strategy 9 from “Mercury in Maine,” a report by the Land and Water Resources Council to the Maine legislature in January 1998, recommends “focus biological research efforts on the effects of mercury on the health of loons, fish and other wildlife with elevated mercury levels.” Emphasis from policy makers and researchers has been on higher trophic level piscivorous wildlife since they are most at risk due to mercury’s ability to bioaccumulate and biomagnify (Scheuhammer 1991, Thompson 1996, USEPA 1997). This interest has facilitated a new initiative by the USEPA-Office of Research and Development’s NHEERL program to investigate stressors (such as Hg) using a focal species (such as the loon) to provide geographically relevant empirical information for science-based policy.

Using Birds as Bioindicators of MeHg Availability

The use of piscivorous birds as indicators of MeHg availability is common (e.g., Thompson 1996, Evers et al. 1998a,b, Wolfe 1998, Wolfe and Norman 1998). We believe piscivorous birds are also useful as general ecological indicators of aquatic ecosystem integrity and of the presence and effects of environmental stressors.

Mercury deposition and MeHg availability is now sufficiently elevated in the Northeast region to cause impacts on wildlife (Welch 1994, Burgess et al. 1998, Nocera and Taylor 1998). Based on the USEPA probability-based sampling efforts in the USEPA’s Region 1 and 2, Yeardley et al. (1998) predicted that 98% of New England’s lakes contained fish with MeHg levels exceeding critical values for piscivorous birds. In corroboration, Evers et al. (1998a) found Common Loons (*Gavia immer*) breeding in the New England region had the highest mean blood Hg levels in the United States, while juvenile loon blood Hg levels were four times those of the designated reference site in Alaska. Further studies on a suite of five piscivorous birds in Maine indicated over 70% of lakes have the capacity to produce MeHg at levels above designated risk categories (Evers et al. 1998b). These studies demonstrate that extensive Hg contamination and MeHg availability exists in Region 1.

Yeardley et al. (1998) found from analyzing 11 metals in fish throughout the U.S. that, “MeHg was determined to be the elemental contaminant of regional concern to fish consumers.” Our study focused on assessing the ecological risk of Hg to a piscivorous bird—the Common Loon. We selected the loon as our bioindicator because of a vast amount of information available on its demographics (e.g., Piper et al. 1997a, Piper et al. 1997b, Evers et al. 2000, Evers 2001), behavioral ecology (e.g., Evers 1993, Nocera and Taylor 1998, Paruk 1999), toxicology (e.g., Evers et al. 1998a, b, Meyer et al. 1998, Scheuhammer et al. 1998a, b), and local breeding population status (Taylor and Vogel 2000).

Mercury Risk to Loons

An estimated 21-37% of the New England Common Loon breeding population have Hg levels that exceed wildlife safety thresholds designated by other studies (e.g., Barr 1986, Scheuhammer 1991, Thompson 1996, Burgess et al. 1998, Meyer et al. 1998). Furthermore, over 60% of abandoned loon eggs collected in New England and New York (n=517) have elevated Hg levels (i.e., 0.5 ppm) according to laboratory studies (Fimreite 1971, Heinz 1979) and 5% have lethal levels (i.e., 2.0 ppm) (Thompson 1996).

These and previous studies documenting exposure in Maine loons (Evers and Reaman 1998, Evers et al. 1998b) predict that impacts are likely. This study was initiated to (1) determine the extent of actual impacts on loons in Maine's lakes and ponds and (2) improve confidence levels on the impact thresholds used to establish risk. We and other collaborators believe relationships exist between high Hg levels and: (1) decreased egg laying capability, (2) decreased egg hatchability, (3) altered parental investment, (4) altered chick behavior, (5) reduced fitness in adults and juveniles, (6) decreased juvenile survival, and (7) reduced lifetime reproductive success. Complementary collaborative studies in the Great Lakes, other New England states, and Canadian Provinces aid in interpretation of our results.

Study Area

BioDiversity Research Institute (BRI) has collected Hg exposure, demographic, and physiological information on New England's breeding loon populations since 1993. Much of this research has been based in the upper Androscoggin and Kennebec River watersheds (e.g., Evers and Reaman 1998, Evers et al. 1998a,b, Evers et al. 200). Because of this knowledge base and some of the highest Hg levels recorded for Common Loons in North America, we have continued our focus on the Rangeley Lakes area for a high resolution study on the potential impacts of Hg to wildlife (Figure 1). In addition to the 44 study lakes and 181 territories in the Rangeley Lakes Study area (including Lake Umbagog), we included 36 other territories from New Hampshire to further develop insight into population level impacts of Hg.

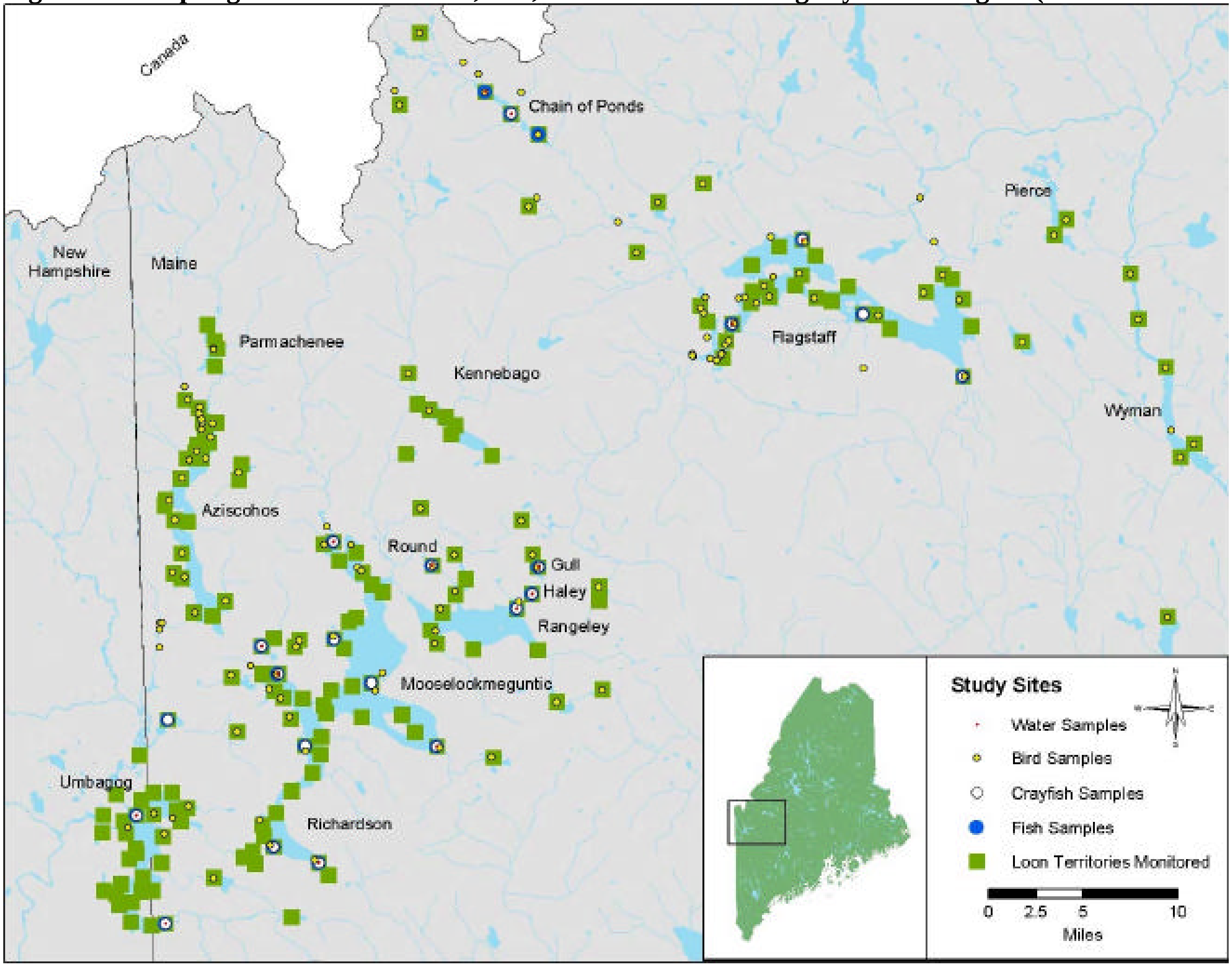
Lakes were classified as natural or those impounded by dams (i.e., reservoirs). We monitored 123 loon territories on seven reservoirs and 58 loon territories on natural lakes (Table 1). Impoundments were defined according to their water management regime by Evers and Reaman (1998): Regulated storage reservoir (RSR) had annual fluctuations greater than 1.5 m, regulated peak reservoirs (RPR) had weekly water fluctuations over 1 m, while regulated full ponds (RFP) were raised lakes managed for minimal water level fluctuations of less than 1.5 m. Drainage lakes had both an inlet and outlet where the primary water source is stream drainage while spring lakes have no inlet but do have an outlet. The water source for spring lakes is the groundwater flow from the immediate drainage area.

Because water level fluctuations from reservoirs impact loon reproductive success more than natural lakes, we only included territorial pairs using rafts floated as part of FERC relicensing. BRI staff monitor loon territories on Flagstaff and Aziscohos Lakes as part of a license agreement by FPL Energy Maine Hydro. Most territories on Aziscohos, Flagstaff, and Wyman have rafts. Raft management has been recently implemented on Mooselookmeguntic and Richardson Lakes. Water level changes on Rangeley and Umbagog are minimal and have minimal impact on loon productivity.

As part of our developing a wildlife criterion value (WCV) for Maine we also included a statistically random sampling effort based on the USEPA's Regional Environmental Monitoring and Assessment Program (REMAP). From 1992-1994, the MDEP conducted a Hg exposure analysis using125 lakes chosen with

REMAP protocols. Of these lakes, those over 60 acres (24 ha) were surveyed and included in this report (Table 2).

Figure 1. Sampling locations for water, fish, and birds in the Rangeley Lakes Region (2001 data in Table 1).



METHODS

Wildlife Criterion Value

Construction of a Maine-based WCV for Hg includes integrating field data with known information. In this first-year, the WCV was primarily based on the Common Loon. The WCV formula from which collection of field data was based on includes data needs for dose-response of Hg at the population level. The WCV used here is from Nichols and Bradbury (1999).

$$\text{Wildlife Criterion Value (WCV)} = \frac{(\text{TD x } [1/\{\text{UFL x UFA x UFS}\}] \text{ x WTA}}{\text{WA} + ([\text{FD3 x FA x BAF3}] + [\text{FD4 x FA x BAF4}])}$$

Whereas:

TD = Tested does from toxicity studies with wildlife species (ug Hg/kg body weight/day)
UFL = The uncertainty factor between the lowest observed adverse effect level (LOAEL) and the no observed adverse effect level NOAEL
UFA = The uncertainty factor between species
UFS = The uncertainty factor between subchronic and chronic levels of impacts
WTA = Average species weight (kg)
WA = Average daily volume of water consumed (L/day)
FD 3,4 = Fraction of diet from trophic level 3 and 4
FA = Average daily mass of food consumed (kg/day)
BAF 3,4 = Aquatic life bioaccumulation factor for trophic level 3 and 4 (L/kg of Hg in fish / Hg in water)

We assessed exposure and hazards based on a large number of color-marked individuals to determine the dose of Hg needed to cause impact. Hazard assessment protocols for behavioral and physiological assays are detailed in Evers et al. (2001). As seen in the formula, uncertainty factors are an important component and we were able to further develop our hazard assessment with a goal to better understand the LOAEL and NOAEL. Species and subchronic differences were not analyzed. Individual bird weights were collected during blood sampling. The average daily volume of water consumed was considered negligible. The fraction of diet from trophic level 3 and 4 was based on Barr (1996). The bioaccumulation factor could then be determined for both trophic levels through measurement of Hg in the water column and then appropriately stratified.

The sampling strategy for assessing Hg exposure in water, fish, and loons in 2001 follows.

Collection of bird tissues for exposure assessment

Blood was drawn from the metatarsal vein through a leur adapter directly into 5-10 cc vacutainers with sodium heparin (green tops) and placed immediately on ice in a cooler. Vacutainers were opened once, 10-14 hours later, to add 10% buffered formalin (1:20 formalin-blood ratio) using USFWS protocols (Stafford and Stickel 1981, Wiemeyer et al. 1984). Each time, formalin was drawn from a sealed container with a new one cc syringe with a measurement precision of 0.02 cc. The vacutainer with blood preserved with formalin was then placed in a refrigerator and not opened until reaching the lab. Whole blood from samples less than one cc was immediately frozen and vacutainers not opened until analysis. Feathers were clipped at the calamus and placed in a polyethylene bag. Methylmercury is locked in the keratin proteins in the feather and is not subject to degradation (Thompson 1996). Feathers were clipped again at a standard location at the the superior umbilicus and cleaned in the lab to remove external contaminants.

When possible, BRI biologists collected whole eggs from nests that had been abandoned, predated or flooded. Eggs were only removed from a site when the adults were no longer incubating them, or they were determined inviable (i.e., strong odor, or indications that eggs were not turned). Eggs were placed in a polyethylene bag and labeled with lake and territory name, date, and collector while in the field. Eggs were then frozen as soon as possible. Later, eggs were measured for length, weight, and volume. Egg volume was measured by water displacement and weighed on an electronic balance to the nearest 0.001g. Egg weight was also measured to the nearest 0.001g. The egg length and width were measured with calipers to the nearest 0.01 mm. Eggs were cut open with a scalpel and the contents were placed into sterile I-Chem® jars (including as much of the egg membrane as possible). The contents were then categorized into one of five developmental stages (Evers et al. In Press).

All samples were labeled in the field within a standard protocol including date, species, age, sex, band or identification number, lake and specific locale, and state. In the field lab, samples were listed on a form and another label was made based on the field form, compared with the field label, and added to the sample (therefore all samples were double labeled). A catalog was developed in the field and a proofed copy accompanied the samples when sent to the analytical lab and again were proofed before preparation for analysis-creating a secure chain of custody of samples. All eggs were adjusted for moisture loss by dividing the total egg weight by the egg volume (Stickel et al. 1973).

Collection of fish for exposure assessment

In 2001, we emphasized the capture of yellow perch (Perca flavescens). Several methods were used to collect the targeted species and sizes. Angling was used to catch large (15-20 cm) and extra large (20-25 cm) size classes of yellow perch. To collect small (5-10 cm) and medium (10-15 cm) size classes of yellow perch we used a 2 m seine net with 32 mm mesh. Fish were measured (weight and total length) and kept in a clean bucket filled with lake water until the day's end when fish were sacrificed, wrapped in plastic and placed on ice until processing later in the evening. Process techniques followed the U.S. Geological Survey's Biomonitoring of Environmental Status and Trends Program (USGS 1999). Whole body analysis ruled out the need for fillets.

Collection of water for mercury exposure assessment

All lake water column sampling was carried out using the USEPA method 1669. Upon arrival at the sampling site, one member of the two-person sampling team was designated as "dirty hands"; the second member was designated as "clean hands." The individual designated as "clean hands" handled all operations involving contact with the sample bottle and transfer of the sample from the sample collection device to the sample bottle. "Dirty hands" was responsible for boat operation and for all other activities that did not involve direct contact with the sample or the sample bottle.

The laboratory provided a clean container filled with reagent water for use with collection of field blanks during sampling activities. The reagent water-filled container was shipped to the field site and handled as all other sample containers and sampling equipment. One field blank was processed per every ten samples (Section 9.4). Sampling personnel wore clean, nontalc gloves at all times when handling sampling equipment and sample containers. The lab provided all sampling equipment.

In addition to processing field blanks at each site, a field duplicate was collected per 10 samples (Section 9.5). Section 9.0 gives a complete description of quality control requirements. Whenever possible, samples were collected facing upstream and upwind to minimize introduction of contamination. Subsurface samples were collected by suction of the sample into an immersed sample bottle. The sample bottles were labeled, double bagged and kept refrigerated. Samples were shipped on ice to the lab within 24 hours.

Techniques and Definitions for Reproductive Measures

In 2001, we surveyed nesting and non-nesting territorial loon pairs on 181 territories from ice-off (early May) until mid-September (Table 1). Surveys consisted of locating loon pairs every 6-8 days from a boat with 10x binoculars, documenting territorial duration, nest attempts, incubation efforts, causes for nest abandonment/failure, and number of chicks hatched and fledged. Human disturbance, evidence of predators, and frequency of intruding loons were also documented at this time. Reproductive information from New

Hampshire was based on a 25-year productivity database from the Loon Preservation Committee (Taylor pers. com.).

We collected four reproductive parameters from each territory: (1) presence of territorial pair, (2) nesting attempts, (3) hatching success and (4) fledging success. Determining whether a loon laid an egg was the most time intensive parameter. Because territories could not always be monitored within our standardized guideline, we could not always confirm whether an egg was laid and therefore we did not use those territories for the nesting pair parameter during our overall productivity analysis. Successful fledging was defined as young loons reaching the age 6 weeks or older. This is consistent with most national loon population monitoring programs.

Laboratory Analysis

Lab analysis for Hg in blood and feathers follow Evers et al. (1998). Analyses of 2001 blood and feather tissues, as well as samples from previous years, were conducted by Dr. Bob Poppenga, Animal Health Diagnostics Laboratory, University of Pennsylvania, New Bolton, Pennsylvania. Lab analysis for Hg in eggs follows Evers et al. (In Press). Analyses of 2001 egg samples were conducted by Dr. Bob Taylor, Trace Element Research Laboratory, Texas A&M, College Station, Texas (TERL). Lab analysis of Hg in fish follow U.S. Fish and Wildlife Service protocols. Dr. Bob Taylor at TERL conducted the Hg analyses of <2001 fish and the Maine Environmental Laboratory, Yarmouth, Maine (MEL) conducted the Hg analysis of 2001 fish. Results from a 20 fish-composite split between MEL and TERL are forthcoming. All biotic Hg levels are for total Hg and are on a wet weight basis.

Dr. Paul Boothe conducted Hg analysis of unfiltered water column samples from 2001. All water samples were analyzed at the Albion Environmental Laboratory, College Station, Texas using USEPA Methods 1631. This method is for determination of Hg in filtered and unfiltered water by oxidation, purge and trap, desorption, and cold-vapor atomic fluorescence spectrometry (CVAFS). This method is for use in EPA's data gathering and monitoring programs associated with the Clean Water Act, the Resource Conservation and Recovery Act, the Comprehensive Environmental Response, Compensation and Liability Act, and the Safe Drinking Water Act. Specific protocols of these analyses follow:

1. A 100-2000 mL sample is collected directly into specially cleaned, pretested, fluoropolymer bottle(s) using sample handling techniques specially designed for collection of mercury at trace levels (Reference 6).
2. Samples are preserved by adding 5 mL/L of pretested 12 N HCl (to allow both total and methyl Hg determination) or 5 mL/L BrCl solution, if total mercury only is to be determined.
3. Prior to analysis, a 100 mL sample aliquot is placed in a specially designed purge vessel, and 0.2 N BrCl solution is added to oxidize all Hg compounds to Hg(II).
4. After oxidation, the sample is sequentially prereduced with NH2OHHCl to destroy the free halogens, and then reduced with SnCl2 to convert Hg(II) to volatile Hg(0).
5. The Hg(0) is separated from solution by purging with nitrogen onto a gold-coated sand trap.
6. The trapped Hg is thermally desorbed from the gold trap into an inert gas stream that carries the released Hg(0) into the cell of a cold-vapor atomic fluorescence spectrometer (CVAFS) for detection.
7. Quality is ensured through calibration and testing of the oxidation, purging, and detection systems.

Statistical Procedures

Mercury concentrations are expressed as arithmetic means because data were normally distributed based on normal probability plot residuals. Homoscedasticity was checked with Bartlett's test, which is sensitive to the normality assumption, and variances were generally found to be similar. Therefore log transformations were not required to stabilize variances for linear relationships. JMP software (SAS Institute, 1999) was used to test various hypotheses using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant different tests if our ANOVA showed significant differences, Student's t-test was used when comparing paired data sets. JMP software corrected for inequity of unbalanced data sets. In all cases, means are given with one standard deviation unless otherwise noted. The level of statistical significance was defined as $p < 0.05$.

Results and Discussions

The following description and interpretation of MeHg risk on the Common Loon is the ninth year of a long-term biomonitoring effort in New England and elsewhere in North America. The construction of this large temporal and spatial database will facilitate and improve national wildlife risk assessments by federal and state government agencies. It also provides the foundation for the Maine-based WCV to be constructed during this 3-year study.

Development of a Maine-based WCV depends on acquiring appropriate data from Maine and neighboring areas. The first task is determining the amount of Hg that causes impacts to wildlife at the population level and to conduct that work in a wild setting that engages everyday natural stressors. The results and interpretation from nine years of Hg exposure in the Common Loon and four years of Hg impacts to this species follow. Exposure is provided within a national context, regional profile, and through a Maine-based random sampling effort. A high resolution effort in the Rangeley Lakes Region provides further insight into Hg exposure but is also the primary location for assessing the impacts of Hg. The hazard assessment effort follows the exposure assessment and documents behavioral, physiological, reproductive, and survival impacts. The risk characterization is based on a random sampling strategy and is thereafter integrated into the test dose component of the WCV formula. Development of the Maine-based WCV finishes the report with information on loon weights, trophic level and proportion of daily food intake, and bioaccumulation factors based on Maine lakes. Uncertainty factors were not emphasized this year. In response, this Maine-based WCV is conservative and is restricted to the Common Loon.

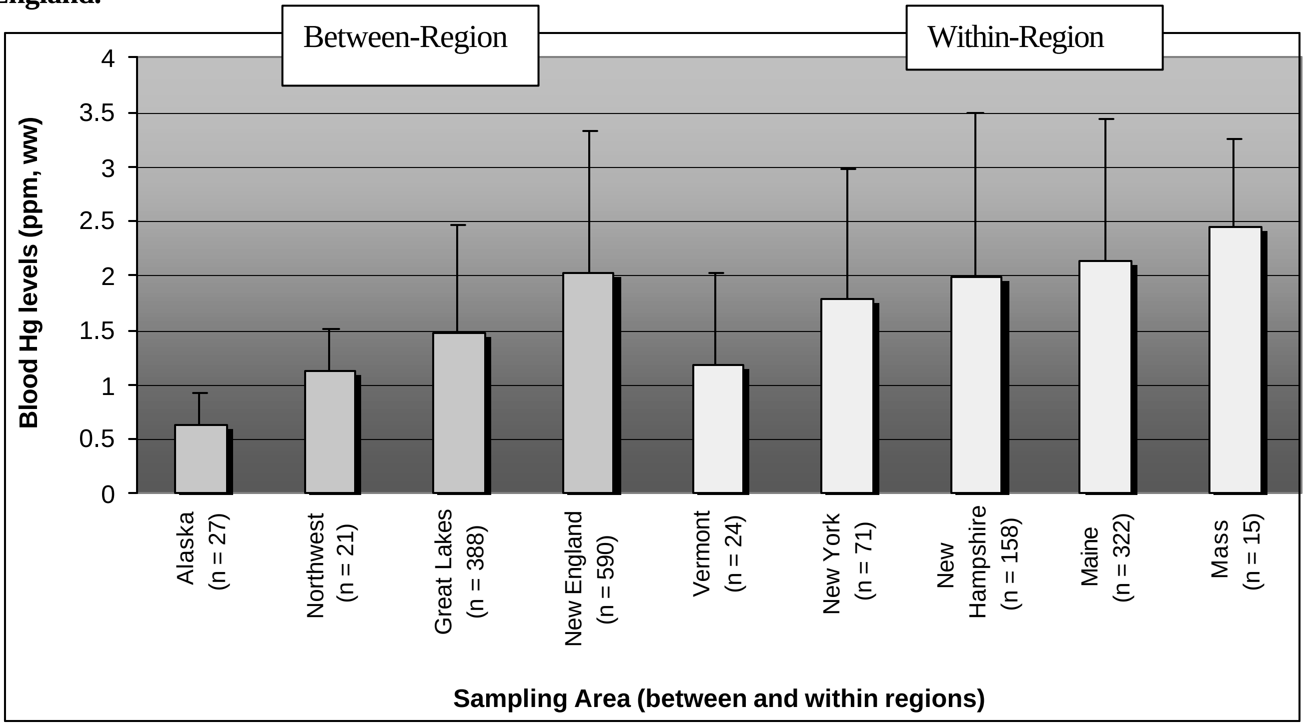
A. Exposure Assessment

1a. Common Loon Mercury Profile – National Context

Sampling of loons and other biota from 1992-2001 indicate geographic differences in MeHg availability. A west to east trend exists across North America with New England having the highest levels (Figure 2) (Evers et al. 1998a). Within-region differences, however, are primarily related to hydrological and biogeochemical factors. Within-region loon blood Hg levels appears to be similar in Maine, New Hampshire, and New York and tend to be lower in Vermont. Particularly high levels of MeHg bioavailability are found in the western Adirondack Mountains of New York, in southeastern New Hampshire, and in several areas of Maine (Figure

3). Because of these factors and potential point sources, a geographic risk assessment using EMAP/REMAP protocols needs to incorporate and characterize sampling areas in a probability-based strategy.

Figure 2. Mean blood Hg levels (+/- SD) of adult Common Loons in U.S. regions and within New England.



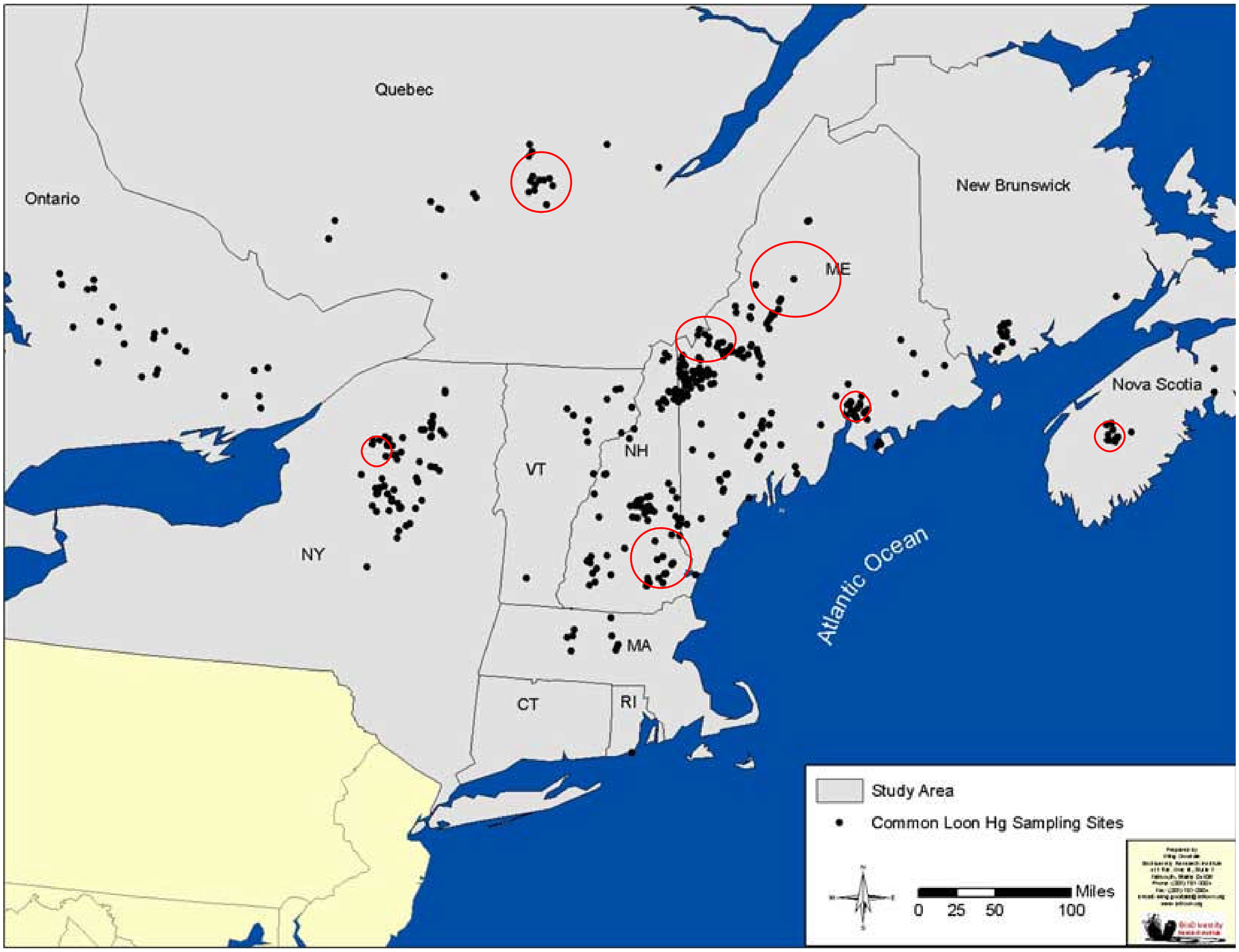
1b. Common Loon Mercury Profile – Northeast Context

The collection of 680 blood Hg levels for the Common Loon across New England, New York and eastern Canada provide excellent context for Maine (Figure 3). Sampling efforts across this region have been primarily opportunistic, although REMAP protocols were used in sampling efforts in Vermont and New Hampshire (Kamman and Engstrom 2002).

In Maine, 322 individual loons were captured and sampled for blood Hg levels from 1994-2001. This large sampling effort has identified several areas in Maine that have higher than average levels of Hg. High Hg areas, or hotspots, have been identified in southern Maine, the western mountain area, and a small area east of Orrington (Figure 3). This latter area is likely related to Hg emissions from a chlor alkali plant. Information based on the Bald Eagle (*Haliaeetus leucocephalus*) (Welch 1994) and River Otter (*Lutra canadensis*) and Mink (*Mustela vison*) (Evers et al. 2002) indicate that north-central Maine has elevated Hg levels that may impact the reproductive success of these and other piscivorous wildlife.

In 2001, 148 blood samples and 47 egg samples were collected in Maine. Actual Hg levels are characterized and integrated into several tables (e.g., Table 1, 2) and figures (e.g., Figure 2, 3) throughout this report.

Figure 3. Distribution of MeHg bioavailability in New England based on loon blood Hg levels with hotspots identified by red circles.



1c. Common Loon Mercury Profile – Rangeley Lakes Region Study Site

There has been a strong emphasis on studying Hg in wildlife within the Rangeley Lakes Region because of interest by the U.S. Fish and Wildlife Service and various members of the Northeast Loon Study Working Group from 1994 to 1997. Elevated levels garnered interest from MDEP in 1997 (Evers et al. 1998) and 1998-2000 (Evers et al. 2001). FPL Energy Maine Hydro also provided support to determine the relationship of biotic Hg exposure and management of reservoirs (Evers and Reaman 1998). These and other efforts now provide one of the more important study sites for assessing the exposure and hazards of Hg on loons and other upper trophic level wildlife.

In 2001, we monitored the reproductive success of loons on 44 lakes with 181 territories. We now have loon blood or egg Hg levels from 121 of these territories (67%). The combination of color-marked individuals with known Hg levels provides a strong basis for determining individual-level reproductive changes related to stressors like Hg. This information can then be transferred to population models.

Table 1. Common Loon territories monitored for overall reproductive success and sampled for Hg exposure.

Lake	Lake Type	# of territory-years monitored	# of territories monitored in 2001	# of territories w/ Hg levels	Low Hg Risk	Mod. Hg Risk	High Hg Risk	Xhigh Hg Risk
Arnold	drainage	2	1	0	0	0	0	0
Aziscohos	Reservoir-RSR	207	21	16	0	7	5	4
B Pond	spring	2	1	1	1	0	0	0
Beaver Mountain	drainage	2	1	1	1	0	0	0
Big Beaver	drainage	2	2	1	0	0	1	0
Big Jim	drainage	10	3	3	3	0	0	0
C Pond	spring	2	1	0	0	0	0	0
Chain-of-Ponds	drainage	7	3	2	0	1	0	1
Cranberry	spring	2	1	1	1	0	0	0
Crosby	drainage	2	1	1	0	1	0	0
Dodge	drainage	2	1	0	0	0	0	0
East Richardson	drainage	5	2	1	0	1	0	0
Flagstaff	Reservoir-RSR	89	21	18	0	5	4	9
Gull	drainage	2	1	1	0	1	0	0
Haley	drainage	2	1	0	0	0	0	0
John’s	spring	2	1	0	0	0	0	0
Kamankeag	drainage	2	1	1	0	1	0	0
Kennebago	drainage	2	8	2	1	1	0	0
Lincoln	drainage	2	2	1	0	1	0	0
Little Beaver	drainage	6	1	1	0	1	0	0
Little Jim	spring	2	1	1	1	0	0	0
Little Kennebago	drainage	2	1	1	0	1	0	0
Little Lobster	drainage	5	1	1	0	0	0	1
Long	drainage	2	1	1	0	0	0	1
Loon	spring	2	1	1	1	0	0	0
Mass Bog	drainage	2	1	1	1	0	0	0
Mooselookmeguntic	Reservoir-RSR	31	19	9	0	9	0	0
Parmachenee	drainage	8	4	3	0	1	0	0
Pepperpot	spring	2	1	0	0	0	0	0
Pond-in-the-River	drainage	5	3	0	0	0	0	0
Pumpkin	drainage	1	1	0	0	0	0	0
Quimby	spring	5	1	1	1	0	0	0
Rangeley	Reservoir-RFP	22	7	6	2	4	0	0
Richardson	Reservoir-RSR	31	16	7	0	7	0	0

Round	drainage	2	1	1	0	1	0	0
Round Mountain	spring	2	1	1	1	0	0	0
Saddleback	drainage	2	2	1	0	1	0	0
Sandy River	drainage	2	1	1	1	0	0	0
Shallow	drainage	2	1	0	0	0	0	0
Sturtevant	drainage	2	1	1	0	0	1	0
Tea	drainage	2	1	1	0	1	0	0
Umbagog	Reservoir-RFP	137	32	25	2	21	2	0
West Richardson	drainage	2	2	1	0	0	0	1
Wyman	Reservoir-RPR	49	7	6	0	0	5	1
Rangeley Area	**(44 total lakes)**	**674**	**181**	**121**	**17**	**66**	**18**	**18**
New Hampshire*	**(33 total lakes)**	**272**	**36**	**38**	**17**	**7**	**3**	**9**
TOTAL	**77 lakes**	**946**	**217**	**159**	**34**	**73**	**21**	**27**

* "New Hampshire" lakes are those outside of the Rangeley Lakes Study area that were not monitored by other biologists during the 2000 season. New Hampshire lakes include Akers (2 territories), Ayers, Baptist, Big Brook Bog, Big Dummer, Brown Owl, Chocorua, Dan Hole, Deering, Duncan, Grafton, Horn, Martin Meadow, Middle Pea Porridge, Massabesic (3 territories), May-Butterfield, Mendums, Millen, Moose, Pontook, Red Hill, Reservoir, Round, Sessions, Spectacle, Sunrise, Swain's, Tower Hill, Upper Kimball, Waukeena, White Oak, Whitton, Willard.

2. Yellow Perch Mercury Profile

We measured Hg in fish to (1) determine Hg pathways to the loon, (2) develop a bioaccumulation factor from water to fish to the loon and (3) understand the relationship among size classes of preferred prey items. Barr (1996) documented the loon's favored prey item was yellow perch. The yellow perch is a relatively ubiquitous species in the Rangeley Lakes Region. Of our 44 lakes, we identified 11 lakes for sampling efforts in 2001. Three lakes were identified as having separate lake basins that could support significantly different fish Hg levels. Lakes within this category include Chain-of-Ponds (3 lake basins), Mooselook-meguntic (2 lake basins), and Richardson (2 lake basins).

A total of 69 composites, each with three individual perch, were collected from 21 territories within 15 lake basins (Table 2). Perch were categorized into four size classes to facilitate trophic level comparisons. Mercury level ranges for each size class are: small (0.04 to 0.13 ppm), medium (0.04 to 0.23 ppm), large (0.08 to 0.28 ppm) and extra large (0.09 to 0.43). Mercury levels reflect the diet of the perch. Based on USEPA (1997) definitions, small and medium size class perch are in trophic level 3 (insect prey specialists) and large and extra large size class perch are in trophic level 4 (fish prey specialists). Significant differences in Hg levels did not exist for intra-trophic levels (TL3 is t=0.4, df=18, p=0.73 and TL4 is t=1.75, df=34, p=0.09) but the Hg level difference for inter-trophic levels was significant (F=13.5, df=71, p=0.0005).

Table 2. Yellow perch Hg (ww, ppm) collected in Maine, 2001 (n=69 composites containing 3 fish/composite).

Lake	Loon Territory	Small (5-10cm)	Medium (10-15cm)	Large (15-20cm)	Extra Large (20-25cm)
Chain-of-Ponds	Long	-	0.230	0.220	0.430
Chain-of-Ponds	Lower	0.050	0.190	0.170	0.200
Chain-of-Ponds	Natanis	0.130	0.100	0.280	-
Flagstaff	Becky Brook	0.050	0.040	0.080	0.220
Flagstaff	Hurricane	0.040	0.140	0.230	-
Flagstaff	Meyer	0.130	0.117	0.190	0.317
Flagstaff	Turner	0.090	0.180	0.190	0.300

Gull		-	0.100	0.130	0.160
Haley		0.050	0.040	0.120	0.120
Mooselookmeguntic	Bemis Cove	-	0.060	0.080	0.090
Mooselookmeguntic	Cupsuptic River	-	0.110	0.180	0.170
Mooselookmeguntic	Students Is.	-	0.030	0.090	0.100
Rangeley	Russell Cove	0.020	0.063	0.105	0.160
Richardson	Black Cat	-	0.050	0.090	0.180
Richardson	Halfmoon Cove	-	0.030	0.080	.07
Richardson	Mill Brook Cove	0.040	0.110	0.120	-
Richardson	South Arm	0.070	0.040	0.120	0.130
Round		0.110	-	-	0.180
Sturtevant		0.050	-	0.140	0.140
Umbagog	Dead Cambridge	-	0.090	0.100	0.100
West Richardson		-	0.110	0.140	0.190

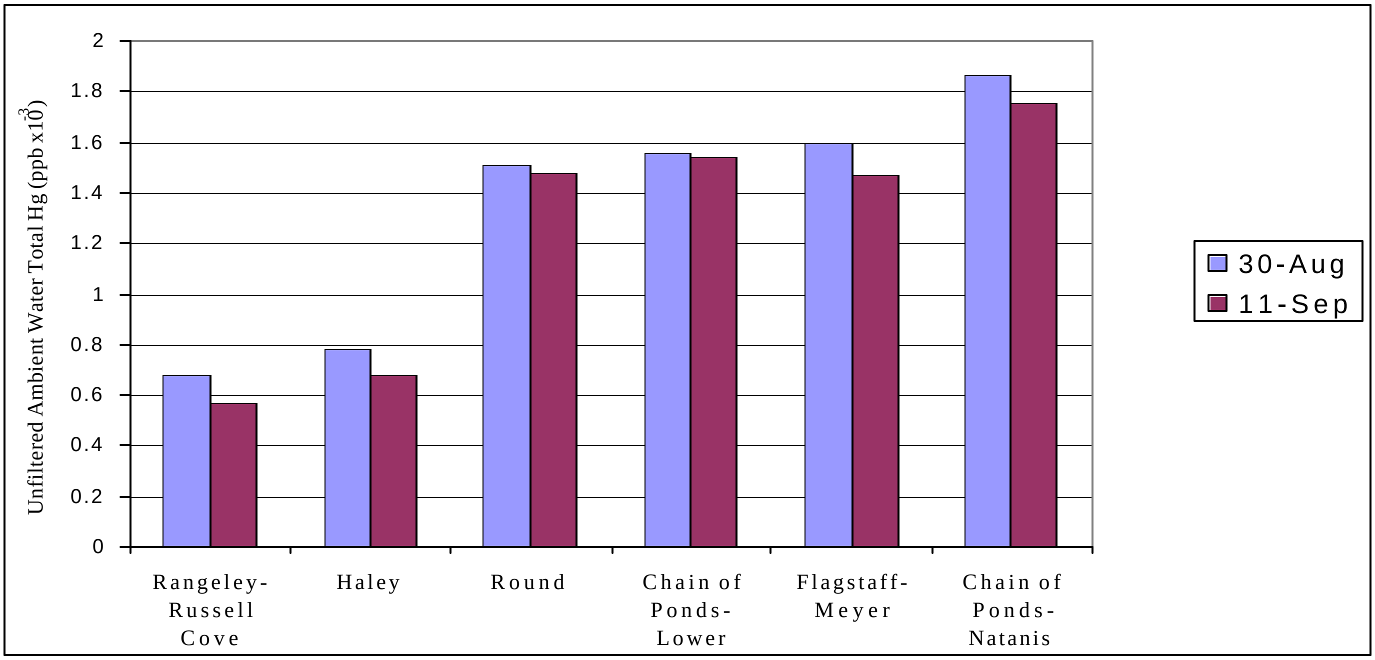
3. Water Column Total Mercury Profile

We choose loon territories that contained yellow perch as a basis for our water column Hg sampling efforts (Table 3). A total of 15 loon territories were sampled. Total Hg in unfiltered water ranged from 0.58 to 2.18 ng/L. Water samples were collected twice for six territories to determine sampling and analytical reliability. Water column Hg levels tended to decline from late August to mid September (Figure 4). However, similarity between sampling events indicates a high accuracy in these measurements.

Table 3. Water column total Hg levels for unfiltered water in the Rangeley Lakes Region, 2001.

Lake / Loon territory	Hg level (ng/L, 29-30 Aug.)	Hg level (ng/L, 11-13 Sept.)
Chain-of-Ponds / Natanis	1.86	1.75
Chain-of-Ponds / Lower	1.56	1.54
Flagstaff / Becky Brook		1.53
Flagstaff / Meyer	1.60	1.47
Gull		0.93
Haley	0.78	0.68
Mooselookmeguntic – Cupsuptic		2.18
Mooselookmeguntic – Bemis		0.69
Rangeley – Russell Cove	0.68	0.57
Richardson – Black Cat		0.57
Richardson – Mill Brook		0.58
Round	1.51	1.48
Umbagog – Leonard Pond		0.94
Umbagog – Dead Cambridge		1.33
West Richardson		1.55

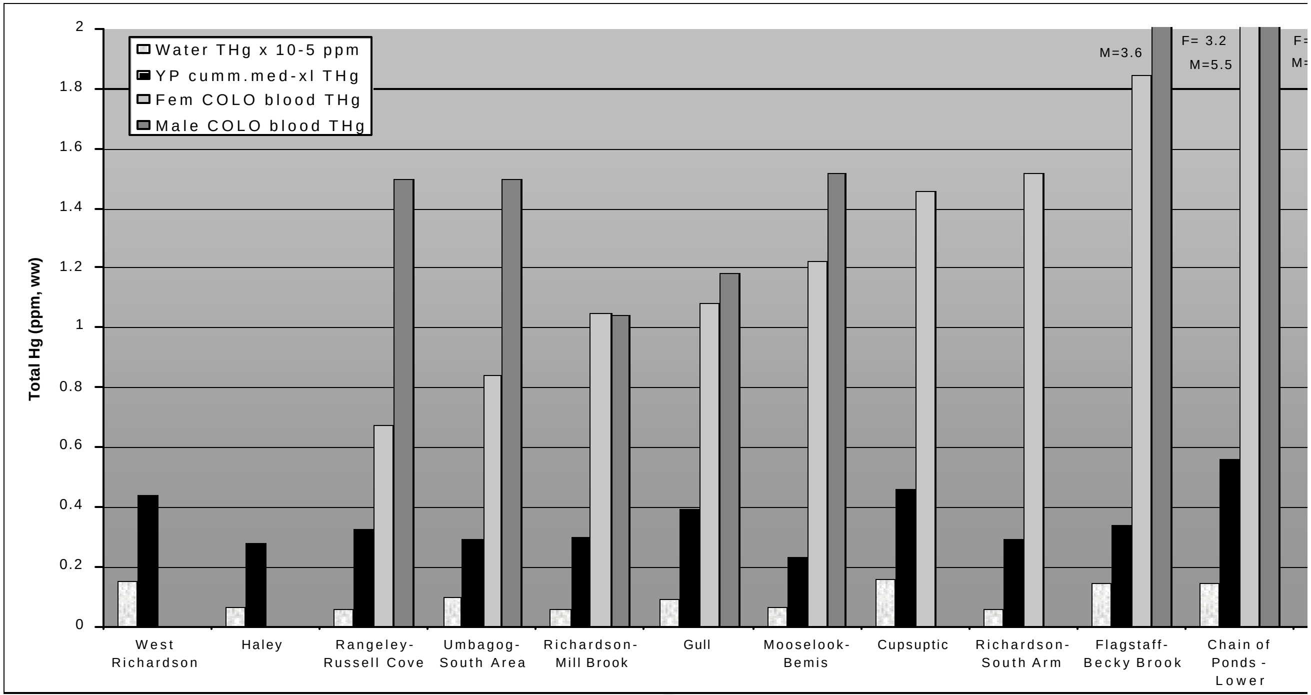
Figure 4. Relationship of repeated-sampling efforts of unfiltered water total Hg levels.



4. Relationship among Hg levels in water, perch, and loons

There were 12 loon territories or lakes where we collected water and fish Hg levels in 2001 (Figure 5). Of these, adult loons were represented in 10 territories (females = 10, males = 8).

Figure 5. Distribution of water, fish, and loon samples collected for Hg levels.

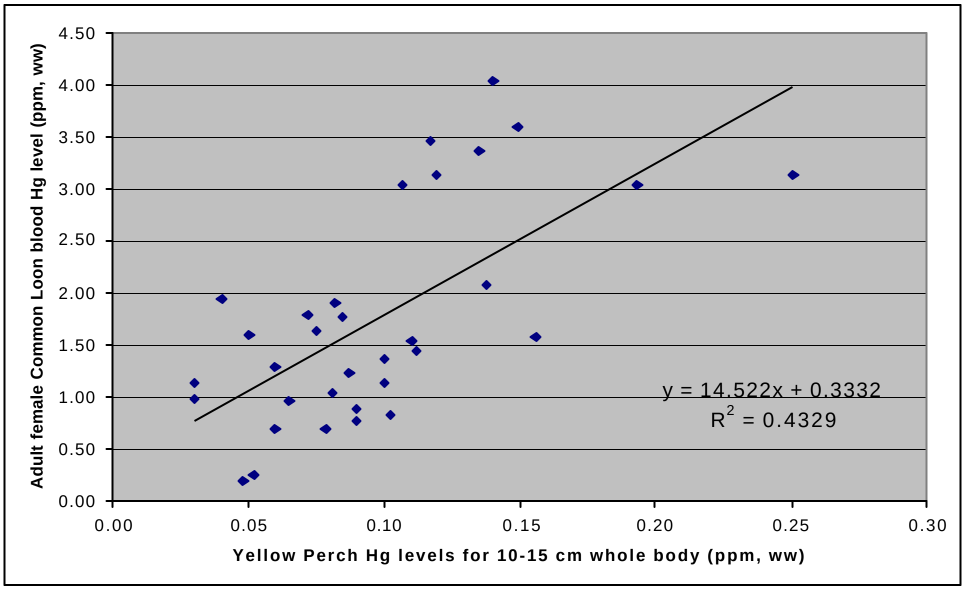


There are important qualifiers to the loon-perch Hg relationship. Although loons prefer perch, diverse fish composition can confound these predictions because loons are more likely to opportunistically forage on other prey items. Secondly, behavioral changes of loons and their prey that are exposed to exceptionally high MeHg levels may alter predictable predator-prey relationships. Studies have shown elevated Hg levels cause fish to change their behaviors in ways that may make them more available to predation (Kania and O'Hara 1974, Weis and Weis 1995, Ososkov and Weis 1996, Schwartz 1998). Significant deviations from normal swimming (e.g., slower sustained swimming times) and escape behaviors (e.g., enhanced zig-zag patterns) were measured in 10-15 cm yellow perch ranging from 0.08 to 0.39 ppm Hg. Kania and O'Hara (1974) found significant behavioral alterations in fish with 0.67 ppm. Nevertheless, Burgess et al. (1998) found a strong relationship between loon blood and similar-size class yellow perch Hg levels (r^2=0.79) and we expected a similar relationship.

We compared perch Hg levels (blocked by size class) with loon blood Hg levels (blocked by sex). To increase sample sizes, perch Hg databases from past years were also included. They include (1) two years of sampling effort led by the U.S. Fish and Wildlife Service (FWS) and Central Maine Power Company in 1996 and 1997 for the Rangeley Lakes area and (2) efforts in New Hampshire that were associated with various FWS projects.

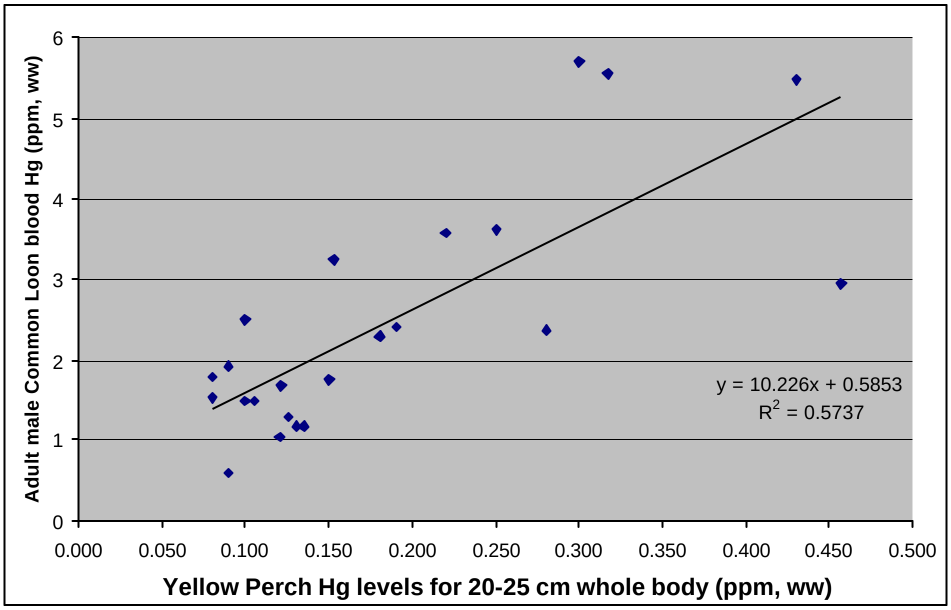
Adult female loon blood Hg levels exhibited weak relationships with small, large, and extra large size classes of perch Hg levels. There was a significant relationship with medium-sized perch (10-15 cm) with 43% of the female blood Hg levels explained (F=23.6, df=31, p<0.001) (Figure 6).

Figure 6. Relationship between adult loon blood (female) and 10-15cm yellow perch Hg levels (total Hg, ppm, ww) in New England.



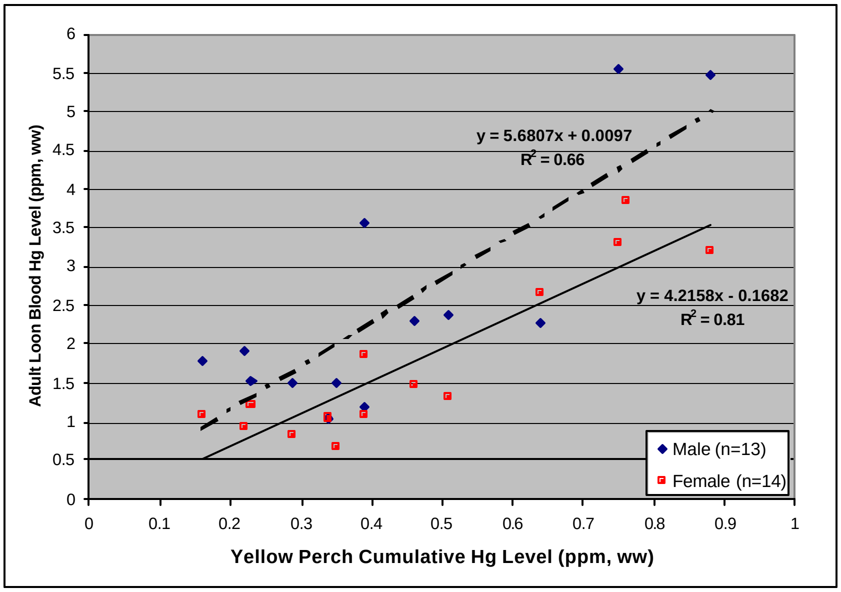
Perch Hg comparisons with adult male loons showed weak relationships with small, medium, and large size classes. Linear regression of extra large-sized perch indicates 57% of the male blood Hg levels can be explained by that size class and species (F=29.6, df=22, p<0.001) (Figure 7).

Figure 7. Relationship between adult loon blood (male) and 20-25cm yellow perch Hg levels (total Hg, ppm, ww) in New England.



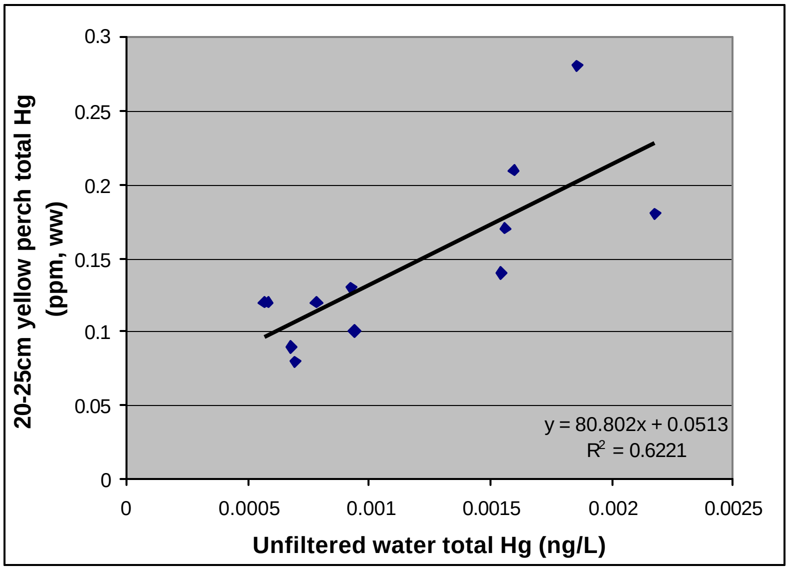
Further analysis of the perch Hg data indicates another approach has merits. We related the cumulative total of Hg levels for the four perch size classes with the blood Hg levels of adult male and female loons. Although samples sizes were smaller, we found a stronger relationship for males (r^2=0.67, F=21.0, df=11, p<0.001) and females (r^2=0.80, F=49.9, df=12, p<0.001) (Figure 8). Because loons vary in body mass and prey size preferences reflect loon size a cumulative approach is appropriate.

Figure 8. Relationship between male and female loon blood and the cumulative product of Hg in four size classes of yellow perch (total Hg, ppm, ww) in New England.



Carter et al. (2001) found a significant relationship with water Hg level and 12 cm yellow perch Hg levels (r^2=0.52). We also found significant relationships, although they varied between the four perch sizes. The relationship of unfiltered water Hg levels and the four perch size classes follow: small r^2=0.46, medium r^2=0.35, large r^2=0.62, and extra large r^2=0.37 (Figure 9). The relationship for our medium perch was less than Carter et al. (2001), although our large perch exhibited a stronger relationship.

Figure 9. Relationship between unfiltered water total Hg (ng/L) and large sized (20-25 cm) yellow perch Hg levels (ppm, ww)



B. Hazard Assessment

We believe investigating multiple levels of biological organization (e.g., genetic, individual, and population) for the Common Loon provides the quantitative benchmarks needed to evaluate environmental stressors like Hg and to thereby contruct a WCV. Detailed results of our hazard assessment for six targeted parameters can be found in Evers et al. (2001) and partly follow recommendations by Peakall (1992). The following are highlights of our four-year hazard assessment.

1. Physiological Relationship with Mercury

The measurement of various blood chemistry parameters and hormones provide a way for assessing an organism's health. They also can be used as biomarkers that can demonstrate the presence and extent of contaminant exposure to an organism and predict potential impacts on that individual (Bensen et al. 1990). For example, Frederick et al. (1997) found a relationship between decreased packed cell volume (PCV) and elevated Hg levels in dosed egrets. Colburn et al. (1993) identified Hg as an endocrine-disrupter and since the loon's body burden of Hg nears the highest tested levels for wildlife in freshwater systems, we measured testosterone and estrogen levels. Corticosterone hormones are released during periods of stress and are being increasingly used as indicators of environmental stressors (Astheimer et al. 1992, Smith et al. 1994), including Hg (Friedmann et al. 1996).

a. Blood Profiles

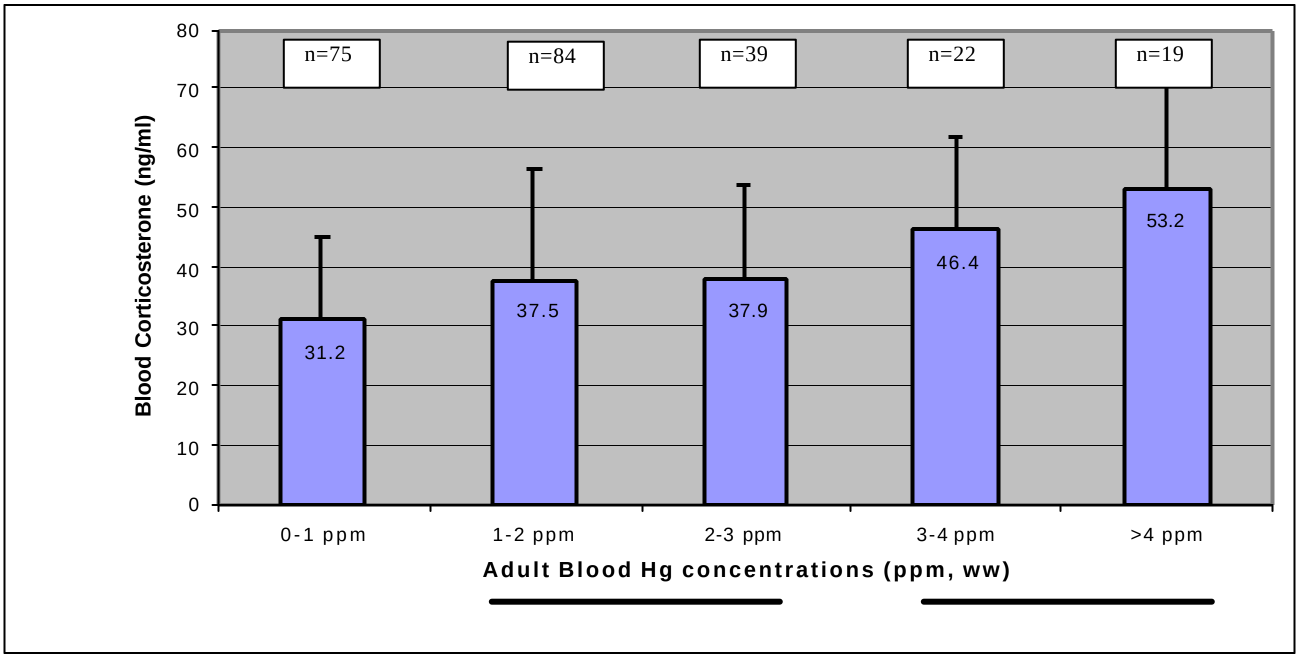
From 1994-99, Tufts University School of Veterinary Medicine (Tufts) and BRI collaborated to develop suitable and logistically simple biomarkers for Hg and to determine reference levels of various hematological parameters in the loon. Over 200 adults and 100 juvenile loons have been sampled. We did not find a significant relationship between elevated Hg concentrations and PCVs ($p>0.05$), white blood cell abundance ($p>0.05$), or white blood cell types ($p>0.05$).

b. Hormones

Although several studies have demonstrated biotic relationships with Hg levels and cortisol stress hormones (Friedmann et al. 1996, Hontela et al. 1992, 1995) those relationships have not been found in birds. BRI and their collaborators at Tufts have recently developed radioimmunoassays for measuring circulating corticosterone levels in loons. Corticosterone is released in response to stressful stimuli and can potentially provide evidence of immunosuppression. There are many confounding factors when comparing corticosterone and MeHg levels, such as handling stress, reproductive stress, and nutritional stress (Sturkie 1986).

During 1995-1999, we collected plasma from 239 adult loons and analyzed the levels of corticosterone. Sturkie (1986) reported ranges of 0.4 to 29 ng/ml for non-stressed birds. We found corticosterone levels to be elevated (i.e., >30 ng/ml) in 88% of the individuals, which ranged from 8.2 to 100.2 ng/ml. Evers (2001) found loon circulating corticosterone levels to be independent of the amount of handling time before taking a blood sample. Therefore, although our elevated levels are partly attributed to the stress of capture, the circulating corticosterone levels we measured are independent of capture and handling times. Loons in the low Hg risk category (0-1 ppm in the blood) represent our reference condition.

Figure 10. Mean concentration of corticosterone versus blood Hg concentrations in adult Common Loons from selected sites in North America (bars represent mean +/- 1 SD)*.



* Those blood Hg concentration categories not connected with a line are significantly different from one another.

When comparing the mean of circulating corticosterone levels with separate categories of increasing one ppm of blood Hg levels, there is a significant increase ($p<0.05$) between low (0-1 ppm) and moderate (1-3 ppm) Hg levels, moderate and high (3-4 ppm), and high and extra high (>4ppm) (Figure 10, Table 4). Therefore, although capture of adult loons with nightlighting methods and the ensuing 30-45 minute handling time does initiate a physiological reaction, stress in our loons with the highest body burdens of Hg does not solely reflect our capture impacts. It appears that blood Hg concentrations have a significant positive correlation with elevated circulating corticosterone levels and that these relationships agree with our established Hg risk categories.

Table 4. Statistical probability matrix for 5 categories of blood Hg concentrations and their relationship with corticosterone levels in adult Common Loons.

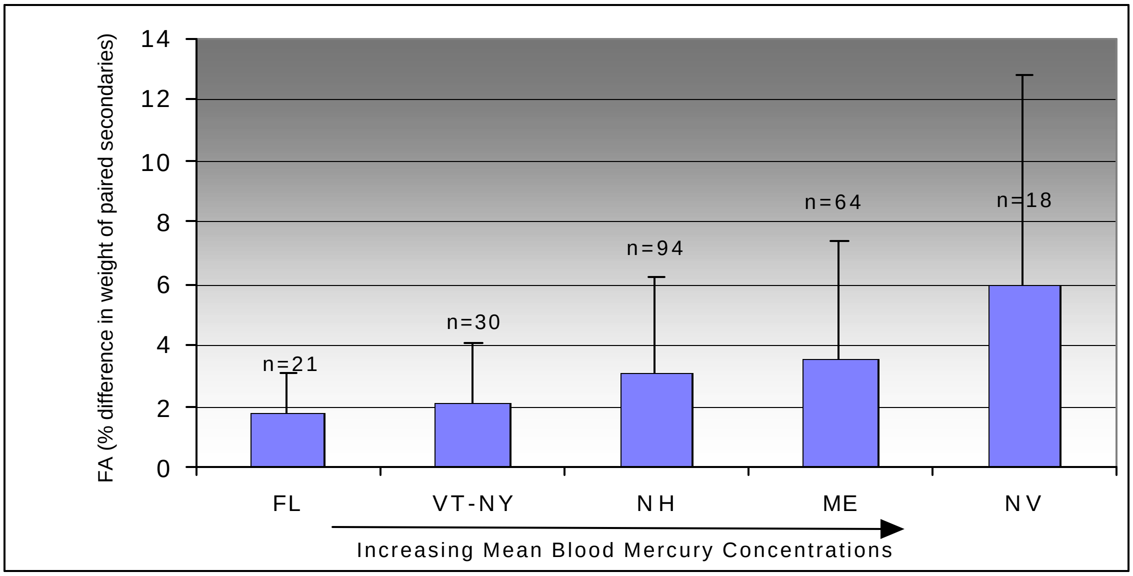
Category	Low	Mod-1	Mod-2	High	XHigh
0-1 ppm	-	p=0.03	p=0.03	p<0.01	p<0.001
1-2 ppm	p=0.03	-	NS	p=0.05	p=0.002
2-3 ppm	P=0.03	NS	-	p=0.05	p=0.002
3-4 ppm	p<0.01	p=0.05	p=0.05	-	NS
> 4 ppm	p<0.001	p=0.002	p=0.002	NS	-

c. Developmental Stability

We measured the relationship between lifetime Hg body burden and fluctuating asymmetry (FA). Clarke (1995) considered the ability of an individual to develop bilateral characters to be one of the best estimates of developmental stability—an indirect measure of fitness. Because feather growth is linked with the very protein reserves that are associated with bound-MeHg in the muscle tissue (Murphy 1996, Scheuhammer 1991), it is likely that remobilization of MeHg coincides with the proteins used for feather formation. Clarke (1995) and Polak and Trivers (1994) suggested FA to be a sensitive measure of long-term body condition and Yablokov (1986) and Moller and Swaddle (1997) both considered FA as a sentinel for subtle environmental perturbations prior to visible effects in population viability.

Analysis of 227 paired feathers collected and measured in 1998-00 indicates breeding populations with higher mean feather Hg concentrations to be significantly more asymmetrical than those populations with lower levels (Figure 11). We compared differences in the weight and length of paired second secondaries and feather Hg levels. Because weights had less measurement error than length, although despite a strong correlation ($r^2=0.86$), we used differences of paired feather

Figure 11. Geographic differences in developmental stability measured through fluctuating asymmetry of Common Loon second secondaries.



weights as measures of FA. Differences in paired feather weights were not significantly correlated with feather Hg levels ($p>0.05$), however, when we pooled individuals according to state as an indicator of Hg stress to breeding populations, New England (Maine and New Hampshire) breeding adults had significantly more flight feather asymmetry than breeding populations with significantly lower feather Hg levels ($p<0.05$). Feather samples from Florida likely represent breeding adults from the upper Great Lakes (Evers et al. 2000), which also have mean blood Hg levels significantly lower than New England loons (Evers et al. 1998a). Migrant loons staging on Nevada's Walker Lake further provide confidence in this analysis. The 26 loon blood samples had a mean Hg level of 3.24 ppm, which is significantly higher than Maine's mean adult blood Hg level of 2.38 ppm. FA in the Nevada loon's feathers was significantly higher than what we found in New England ($p<0.05$).

It appears that the loon's remiges are a sensitive indicator of FA and the relationship of FA with high Hg risk breeding loon populations potentially makes this bioassay technique important for monitoring aquatic integrity. Although other stressors may disrupt developmental homeostasis, and genetic diversity (especially in the loon, e.g., Dhar et al. 1997) may predispose some populations to having greater FA than others, this technique is as an excellent "catch-all" benchmark for predicting subtle environmental stressors.

2. Behavioral Relationships with Mercury

a. Known behavioral relationships with Hg risk

Relating behavior with environmental toxins is a useful indicator of sublethal effects (e.g., Doving 1991). Detection of behavioral abnormalities related to environmental stressors such as Hg provide insight into the most vulnerable behavioral and physiological mechanisms of nesting and chick-rearing that could ultimately impact reproductive success, chick survival and adult survival. Although Nocera and Taylor (1998) and Counard (2001) found subtle but significant behavioral differences related to Hg in loon chicks, similar relationships have not been previously quantified in adults.

b. Geographic and gender differences in behavior

Loon behavior data collected in the Midwest showed few significant differences in parental roles and parental effort during pre-incubation, incubation, and post-hatching periods (Evers 1994 [250 hrs], Mager 1995 [1,400 hrs], Gostomski and Evers 1998 [120 hrs], and Paruk 1999 [4,200 hrs]). We have used these studies as the baseline comparison for the nesting and gender components of our Rangeley Lakes Region study. Behavioral differences within territorial pairs on our Maine study sites had a greater tendency to differ than those studied in the Midwest (Evers et al. 2001).

c. Nesting Period: Behavioral relationships with Hg risk

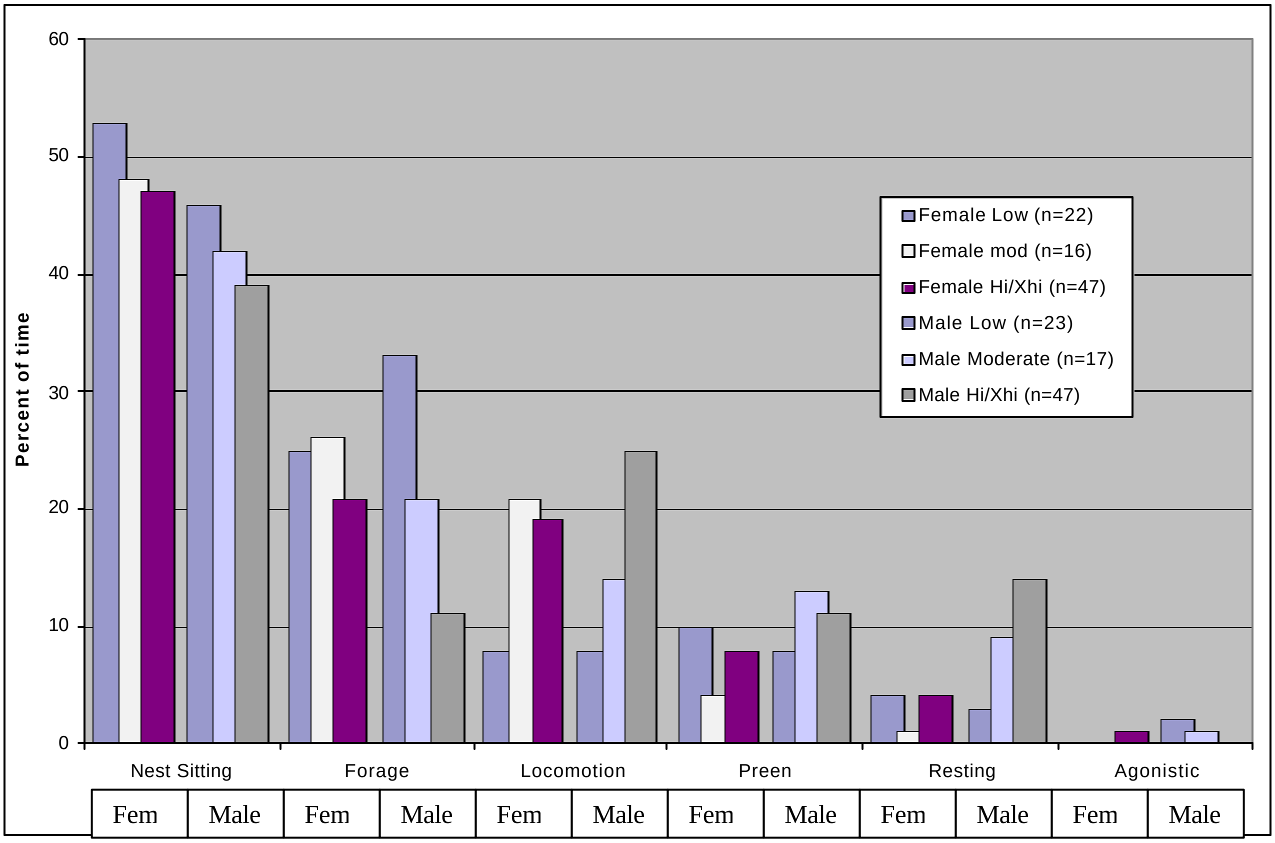
Time spent on the nest is a key behavior for detecting abnormalities related to MeHg body burdens (Figure 12). In this study, we quantified adult behavior through time activity budgets (TABs) during three distinct breeding periods: pre-nesting, nesting and post-nesting.

We have documented several cases where high-Hg males have not properly incubated the eggs. In other studies, Hg has a negative impact on egg laying in loons (Barr 1986, Burgess et al. 1998) and other species (Heinz 1979). In 1999, we reported on aberrant behavior in a high Hg territory. Because incubation is equally shared in loons (Evers 1994, Mager 1995, Paruk 1999), and since male Common Loons are known to have higher levels of Hg than females (Evers et al. 1998a) this discrepancy is notable.

Precedence for measurable Common Loon adult behavior abnormalities has been documented during incubation in past years, and evidence indicates reproductive impairment is associated with high mercury exposure (Barr 1986, Burgess et al. 1998, Evers et al. 2000). This suggests elevated Hg concentrations can have an effect on adult behavior and ultimately reproductive success. Some behavior abnormalities are difficult to quantify or are not necessarily adequately represented using time-activity budgets. Cases of atypical behavior were recorded for individuals identified as having “extra-high” exposure to Hg. These qualitative observations likely represent cases of acute behavioral abnormalities in relation to mercury and can be found in Evers et al. (2001).

We compared the percent time spent by nesting loons in six behavior categories by Hg risk category and gender (Figure 12). We have combined the high and extra high risk categories for this analysis due to insufficient sample size in the high-risk category. This combined category will be referred to as “high” herein. The following describes significant differences in the percent time nesting loons spent in various behaviors by gender and risk category, representing an analysis of behavioral and gender differences across three Hg exposure categories.

Figure 12. Average percent of time spent by adult male and female Common Loons during the nesting period in four Hg exposure categories*.



* Sample size reflects number of individuals

Nesting Period: Nest Sitting

Nest sitting females and males display similar patterns between risk categories. High Hg loons spent less time nest sitting than did moderate-risk individuals, which spent less time nest sitting than low risk loons. When comparing the genders in the behavior of nest sitting, males spent less time than females in every risk category (Figure 12). Males and females at low Hg risk spent 99% of the time incubating eggs; leaving the eggs unincubated for only 1% of the time sampled. Loons in the moderate risk category incubated for a total of 90%, leaving the eggs unincubated for 10% of the time. High risk males and females spent 86% of their time nest sitting, leaving the eggs unincubated for 14% of the time sampled. Unattended eggs have a higher probability of being predated by avian or mammalian predators, which likely results in a higher incidence of nest failures.

Nesting Period: Foraging

When analyzing the percent time males spent in other behavior categories, the stepwise pattern observed between risk categories in nest sitting is consistent with that of foraging. Males follow a more dramatic downward step pattern for foraging than females: high Hg loons spent less time foraging than did moderates, which spent less time foraging than the low risk loons. Evers (1994) reported males and females to forage 34% and 36%, respectively, which is similar to low risk males in Maine (33%). High and moderate risk males in our study, however, spent 11% and 21% of their time, respectively, in foraging behavior. Gostomski and Evers (1998) reported on time-activity budgets of loons nesting on Lake Superior, likely representing the most ideal conditions for turbidity and prey abundance. They reported females to forage11% during the nesting period. Although we have not controlled for the confounder of turbidity in our study, we feel that time spent foraging should lie between the levels given by Evers (1994) and Gostomski and Evers (1998). Other studies, such as Bouton et al. (1999), found a significant relationship between Hg and the mean time necessary to capture fish. Dosed Great Egrets (*Ardea albus*) (0.5mg methyl HgCl/kg) were found to be consistently slower at capturing fish than controls. Significant effects of age and sex were also reported, and dosed birds were significantly less likely to eat fish presented to them (even in cases where they caught them) in both camouflage and contrasting pool background settings, potentially suggesting an impact on appetite and/or metabolism. The effects and differences reported with loons in this study agree with preliminary findings by Frederick et al. (1997), who suggests a debilitating effect of Hg on vision in birds. We feel that there is strong evidence toward a negative relationship between Hg and foraging behavior and efficiency.

Nesting Period: Preening, Resting, and Locomotion

The relationship of Hg risk with other behaviors is detailed in Evers et al. (2001).

d. Post-hatching Period: Behavioral relationships with Hg risk

Once chicks hatched, TABs were conducted on the entire loon family. Because loon chicks molt into their next downy stage at approximately two weeks and retain downy feathers over half of their body until 5 ½ weeks of age, we separately investigated the behavior of adults according to these time periods. This is consistent with Nocera and Taylor (1998) and Counard (2001), who recognized 1-12 days (downy young, or DY) and 13-40 days (small young, or SY) as chick age periods.

Post-hatching Period: brooding

Adults in the 1-12 age period spent the highest percentage of their time brooding (40-60%), while adults in the 13-40 age period appeared to shift previous time spent brooding (35-40%) towards foraging for chicks. Time spent brooding 1-12 and 13-40 day-old chicks showed an increasing trend as Hg levels increased (Evers et al. 2001). Linear regression of this data found a significant positive relationship between the percentage of time male and female loons spent brooding 1-12 day-old chicks and blood Hg levels ($r^2 = 0.312$, $p = 0.037$). The percent of time both adults spent brooding was not significantly correlated with blood Hg in the 13-40 day period ($p = 0.463$), presumably because of the shift in time spent to other behaviors such as foraging for chick. We also measured 5 subcategories within brooding behavior itself (locomotion, drift, preen, underwing, and on-back), and found adults spent significantly less time locomoting while in brooding behavior than low Hg risk loons ($p<0.05$).

As this and other studies such as Bouton et al. (1999) seem to indicate, gender is often an important and significant factor in relation to the effects of Hg on behavior. In most cases, males are more impacted by Hg in behaviors such as foraging than females. This is likely because of the larger males’ tendency to take larger fish. Further analysis of the relationship between parental behavior and Hg are detailed in Evers et al. (2001).

Post-hatching Period: foraging

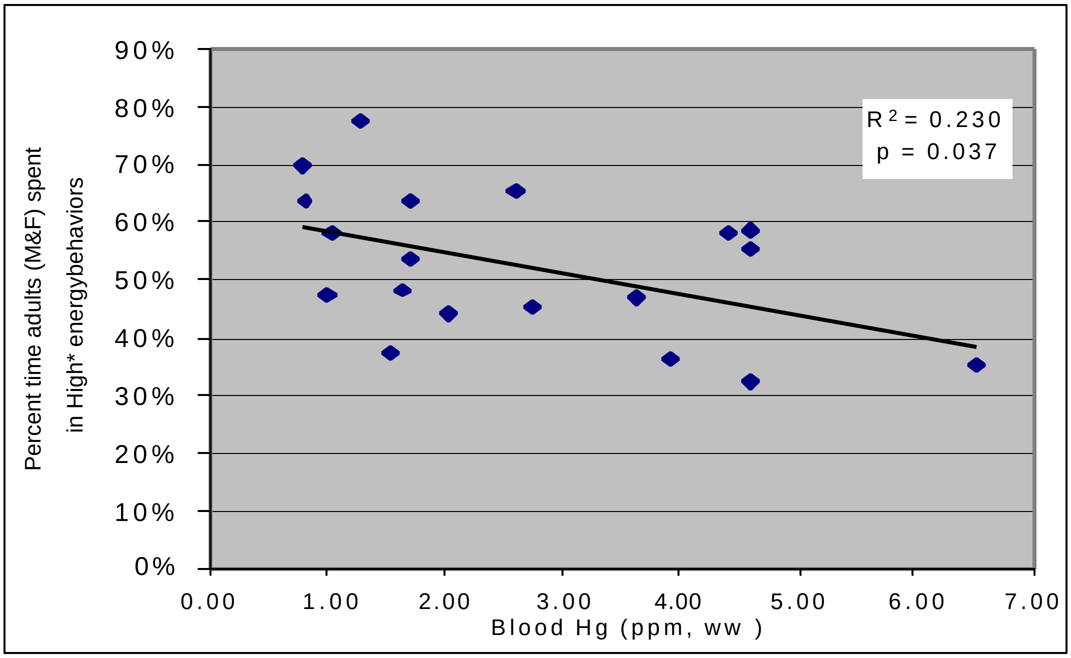
Linear regression analysis for data in both age groups reveals a significant negative correlation between percent time spent by adults foraging for chicks and chick blood Hg levels ($r^2 = 0.265$, $p = 0.024$).

This finding agrees with those reported in the literature. Bouton et al. (1999) reported significant relationships between mercury and motivation of low dosed (0.5mg/kg) juvenile Great Egrets to hunt prey, as well as an increased mean time necessary to capture fish and decreased tendency to eat prey items. Counard (2001) reported a significant relationship between chick begging and mercury, which may be related to a compromise in the adults' ability to adequately meet energetic demands of chicks while foraging. Mercury has also been implicated in impairing learning and physical abilities (Inouye et al. (1985) and Burbacher (1990) in Bouton et al. 1999), as well as reduced motor skills due to damage to the cerebellum and cerebrum (Wolfe (1998). Frederick et al. (1997) reported on preliminary data from a dosing study that suggests a debilitating effect of Hg on vision in birds. Frederick et al. (1997) cites other studies that have linked alterations in photoreceptor function with Hg (Fox and Sillman 1979, Gitter et al. 1988).

e. Behavioral Relationships: mercury and energy expenditure

As previously mentioned in discussions of foraging, other studies have reported relationships between Hg and decreased activity levels (increased lethargy), motivation to hunt, and thermoregulation (Heinz 1996, Thompson 1996, Bouton et al. 1999). Our behavioral findings point towards a negative relationship between blood Hg and high-energy behaviors such as foraging. Conversely, the percentage of time adults spent in behaviors associated with lower energy demands (such as brooding, and resting) appeared to increase with blood Hg and Hg exposure category. To further address this tendency, we separated all behaviors of adults brooding 1-40 day-old young into these two categories based on our current perception of their energetic demands. Foraging for chick, foraging for self, locomotion, preening and agonistic behaviors were grouped together in the "high" category, while brooding and resting (the sum of drift and sleeping) behaviors were categorized as low. We found a significant negative relationship between Hg and the percent time adult male and female loons spent in high energy behaviors while brooding 1-40 day old young ($r^2 = 0.230$, $p = 0.031$; Figure 13). Our findings are similar to Bouton et al. (1999), who reported a general tendency for dosed Great Egrets to spend less time walking, pecking and flying than controls. More important, they similarly report a "negative relationship between mercury dose and the amount of time spent in active, energetic, and maintenance behaviors." We believe that this concept may be the underlying explanation for many of the relationships discovered among specific behaviors and Hg in this study.

Figure 13. Average time adult male and female loons spent in high energy behaviors while brooding 1-40 day-old chicks related to mean blood Hg levels in Maine 1998-00.



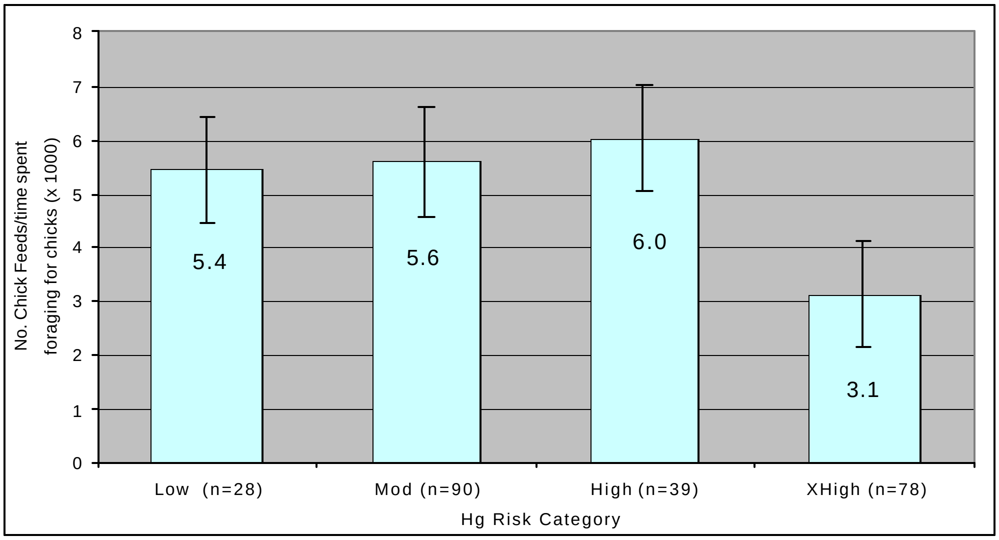
f. Adult Behavior Event Analysis

Few studies have used behavior events to give insight into behavioral impacts of Hg on Common Loons (Counard 2001, Nocera and Taylor 1998). These counted behaviors are easily defined and quantified in the field, thereby minimizing observer and sampling biases (Evers et al. 2001). We believe that some behavior events can give us helpful insights into subtle behavioral differences between risk categories.

Chick Feed Events

The act of feeding young is perhaps one of the most important components of parental care. This is especially true in the first eight weeks after hatching, when the young are most dependent on the adults for food (Barr 1986). Chick-feeding events by adult male and female loons brooding 13-40 day old chicks was significantly lower in the highest risk category compared to lower ones ($p<0.05$) (Figure 14).

Figure 14. Comparison of average chick feeding event behavior by male and female Common Loons brooding 13-40 day old chicks in 4 Hg exposure categories.



Dive Events

Olsen et al. (in prep.) used adult dive event data from this study to address potential impacts of mercury on foraging behavior. Non-parametric testing found a significant difference among diving frequencies of Common Loons of various exposure levels (H = 8.75, df = 3, p = 0.033), and a positive correlation between the diving frequency and mercury risk ($r^2 = 0.136$, df = 249, p = 0.032). Olsen et al. suggest that since Hg is known to inhibit heme (Marks 1985), it lowers the oxygen carrying capacity of the blood, thereby limiting the duration of dives, increasing the frequency of dives necessary to meet caloric needs of themselves and their young. Counard (2001) reported a negative relationship between time loon chicks spent diving and Hg (p = 0.009, $r^2 = 0.32$). Other studies such as Bouton et al. (1999) presented significant findings of a relationship between mercury and the mean time necessary to capture fish. Dosed Great Egrets (0.5mg methyl HgCl/kg) were found to be consistently slower at capturing fish than controls. Significant effects of age and sex were also reported, and dosed birds were significantly less likely to eat fish presented to them (even in cases where they caught them) in both camouflage and contrasting pool background settings. Findings in this and other studies suggest that mercury may interfere with foraging behavior, vision, and coordination.

g. Adult Behavior Summary

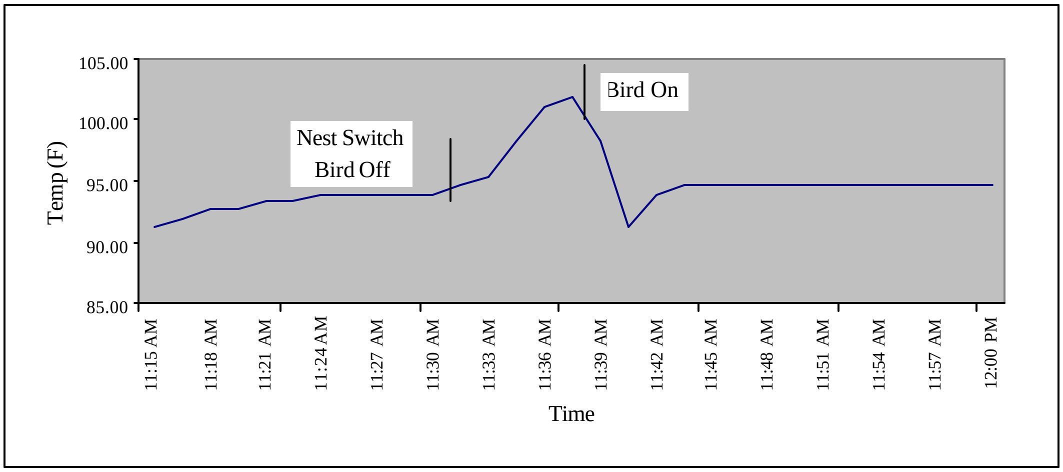
The analysis of 1998-2000 adult loon behavior data indicates a relationship between Hg and several aspects of loon behavior. Stepwise patterns were observed in several behavior categories among loons in Hg risk categories in all nesting stages. Regression analysis on several of these behaviors revealed statistically significant relationships between blood mercury and behavior. The results of this study indicate a relationship between high Hg risk situations and (1) less time spent nest sitting, (2) specific cases of abnormal incubation behavior patterns, (3) less time spent foraging for young and themselves, (4) increasing time spent brooding and resting, (5) increased dive frequency while foraging, (6) behavior impacts biased towards males.

These findings reveal an underlying tendency for mercury to negatively impact the amount of time adult loons spend in high-energy behaviors, thereby shifting time spent into behaviors with lower energetic demands. These behavior alterations could be the precursor of decreasing reproductive performance, chick survival and adult survival.

h. Temperature Dataloggers as measures of adult incubating behavior

Because of known incidences of high Hg risk males exhibiting abnormal incubation patterns, we have attempted to fully measure time spent over several consecutive 24-hour periods. Temperature data loggers were placed in 12 nests during 1998, for a total of over 1,600 hours of monitoring and in four different nests in 1999 for a total of 400 hours. Both nest and ambient temperatures were monitored on 8 of the 12 sites in 1998 and all sites in 1999. A clear example of nest switching as indicated by the data loggers occurred on the Chain of Ponds – Upper territory on June 24 (Figure 15). The nest temperature rose after the loon left the nest, as the nest was in direct sunlight. The nest temperature then declined seven minutes later due to the water and increased aeration of egg turning associated with the arrival of the loon's mate.

Figure 15. Nest temperature (F) for a Common Loon pair, June 24, 1998.



An average of the daily nest temperature (7 am to 10 pm) of 8 data sites was 81.04° F, with a correlative average ambient temperature of 67.56° F. Average nighttime (10 pm to 7 am) nest temperature was 77.35° F, with a correlative ambient temperature of 59.16° F.

An analysis of diurnal and nocturnal temperatures on 8 lakes indicates individuals with high Hg levels may have a higher disparity of daily nest temperatures. Initial data (n=3 nests) show low risk individual loons have an average of 2.09° F difference between diurnal and nocturnal nest temperatures. Territories with a high risk to Hg (n=3 nests) have a daily nest temperature fluctuation of 4.43° F. The use of these temperature data-loggers is promising and in time would provide a greater confidence and accountability of 24-hour incubation patterns by breeding pairs.

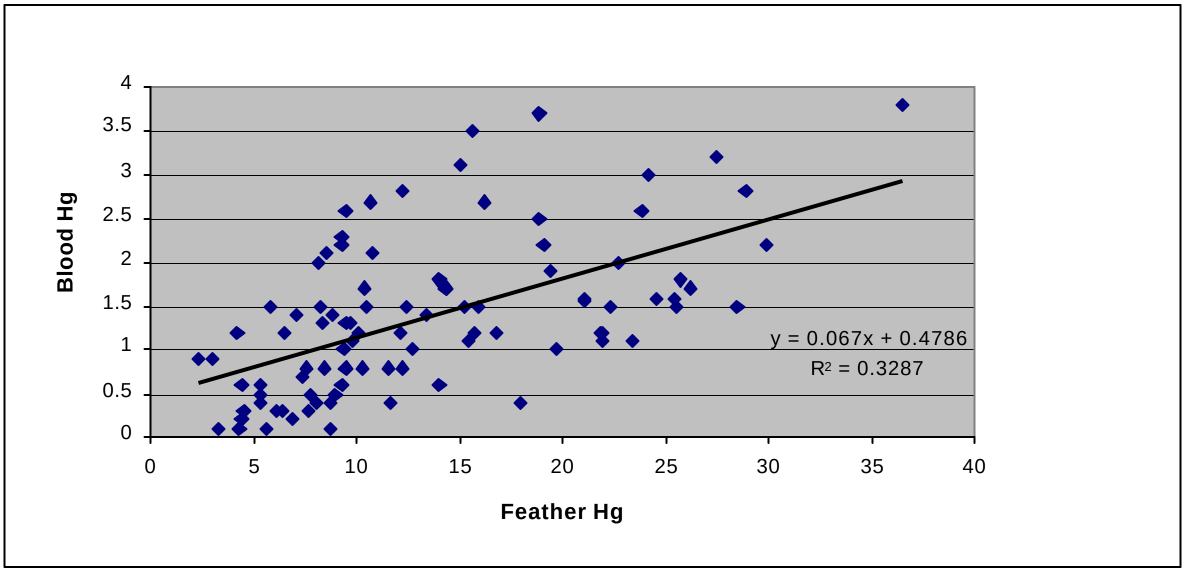
3. Survival Relationship with Mercury

Adults and juveniles show increasing levels of Hg in their body burdens over time (Evers et al. 1998a, BRI unpubl. data). Although birds have natural defense mechanisms for depurating (e.g., feathers), demethylating (e.g., liver and kidney), and sequestering (e.g., egg) Hg (Thompson 1996), high-risk individuals accumulate more Hg than they are able to annually regulate. Excess Hg binds to protein in the muscle tissue and remobilizes during stressful events. Feather molts are energetically demanding, particularly the full remigial molts that loons experience for two weeks during the winter. Because muscle protein reservoirs are associated with feather protein (Murphy 1996), the remobilization of proteins during feather molt partly reflects the available body burden of MeHg in an individual loon.

a. Adult Loons

We found a significant amount of this muscle-bound MeHg originated from prey during the breeding season. In New England loons, there was a significantly positive relationship between blood and feather Hg levels (r^2=0.32, p<0.01) (Figure 16). In past studies, we did not find a significant relationship in breeding loons in the Great Lakes, Pacific Northwest, and Alaska (Evers et al. 1998a).

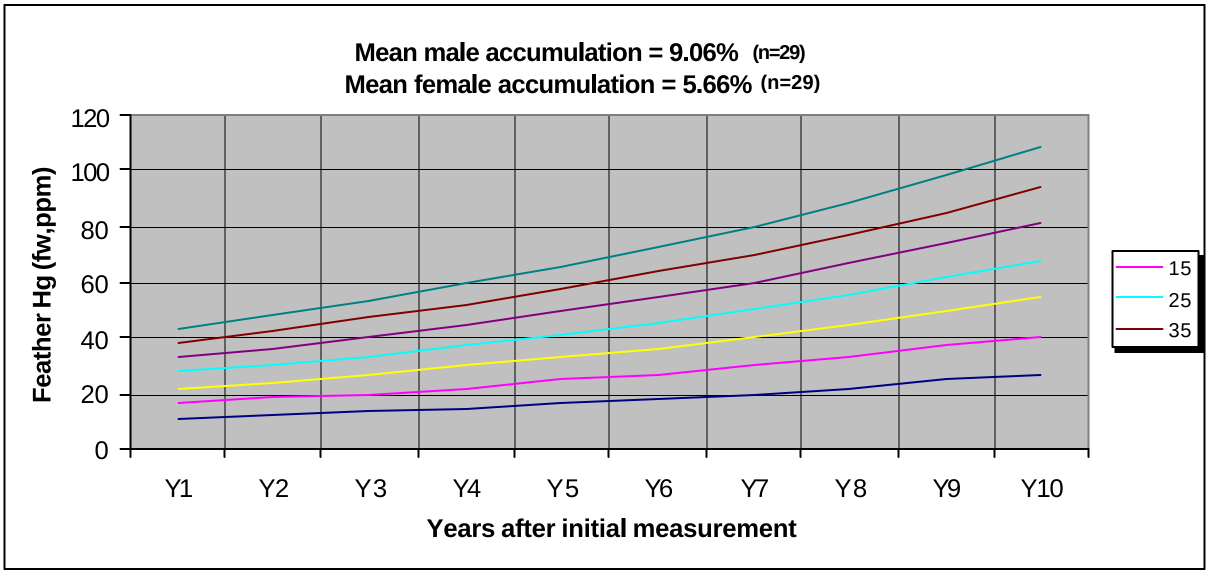
Figure 16. Relationship between blood and feather Hg levels (ppm) in New England Common Loons.



Therefore, the feathers grown during the winter and sampled during the breeding season are indicators of chronic body burdens of Hg. Feather samples collected from recaptured adult loons now indicate this bioaccumulation of Hg is measurable over time and in high-risk populations significantly increases annually. Adult loons were recaptured over the past 1-5 years from Maine lakes. Of 15 males representing 29 accumulation-years, 23 (79%) of those years showed an increase. The mean annual accumulation rate in males was 9.06%. Of 8 females that were recaptured, representing 29 accumulation-years, 21 (72%) of those years showed an increase. The mean annual accumulation rate for females was 5.6%. Male accumulation rates were most likely higher than females because of the females' ability to sequester Hg in eggs (Kambamandi-Dimou et al. 1991) and the tendency to eat smaller prey than males (Evers and Reaman 1998). The body mass of males average is 23% larger than females in New England.

The mean feather Hg levels in Maine's male loons is 14.8 +/- 8.2 ppm (n=87) and in females is 9.8 +/- 5.0 ppm (n=87). A temporal extrapolation using an accumulation rate of 9.06% for a male with 15ppm of Hg in its feather places that individual at high risk and potential impacts in 3 years (i.e., 20 ppm) and probable impacts (i.e., 35 ppm) in 7 years (Figure 17). Although female Hg bioaccumulation rates are lower ($p<0.5$) this rate still only increases the female's reproductive expectancy two years. Because loons are K-selected species, long-term impacts on their reproductive success can potentially have severe population effects.

Figure 17. Bioaccumulation of Hg measured in feathers of recaptured adult loons.

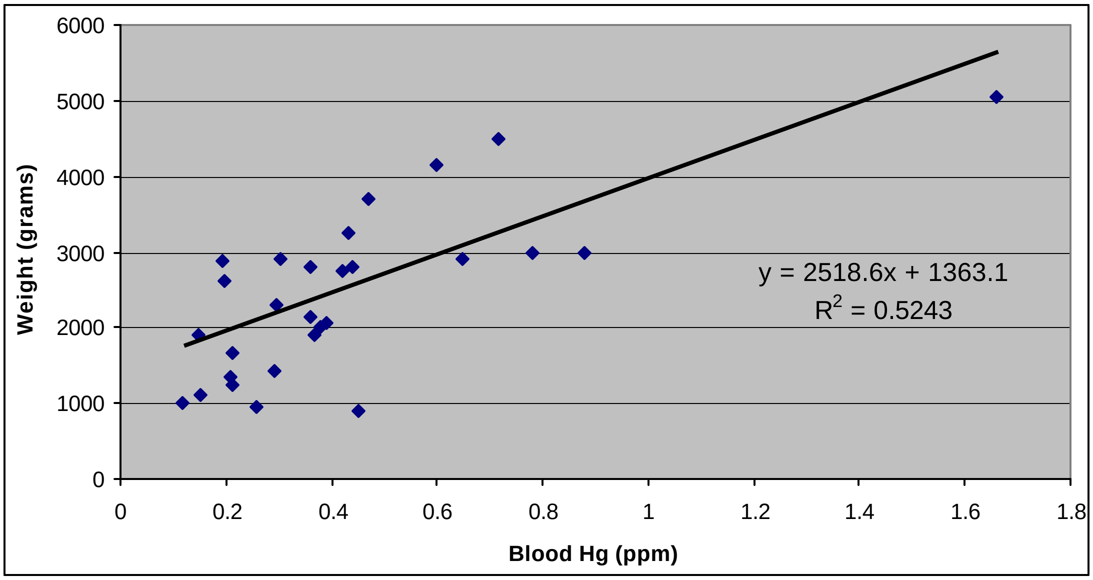


b. Juvenile Loons

Although we have shown environmental Hg levels in Maine impact hatching success, there are also potential impacts on juveniles if they should hatch into a high Hg territory. We suspect that loons in Maine have lower growth and survival rates that are related to the uptake of Hg from their prey. Once a juvenile loon has fully grown feathers, its body burden of Hg can no longer be depurated and begins to accumulate in its blood, brain, organs, and muscle (Frederick et al. 1997). Young loons have three feather molts before attaining a juvenile plumage. From hatching to 12-14 days a black downy plumage covers the chick's body and that is replaced by another downy, but brown plumage lasting for 3-4 weeks. At six weeks many of the contour feathers have replaced the downy plumage, which can only be found on the nape, neck, and flanks. Flight feathers begin to sheath at four weeks and are fully sheathed by 12 weeks. Rate of primary growth is consistent from 5-11 weeks, while overall growth rates begin to fall at 10 weeks (Barr 1996).

We captured 3-12 week old loons on Flagstaff and Aziscohos Lakes from 1994-99 and found a significant correlation ($p<0.01$) between blood Hg concentrations and weight on these high Hg lakes (Figure 18). Although loon weights vary with nutritional and physical stress due to sibling rivalry and habitat quality, we used weight as an indicator of age. Therefore, as young loons aged on their natal territories, Hg levels significantly increased ($p<0.01$). Juvenile loons from lakes with lower risk to MeHg availability also exhibited significant increases but were more weakly correlated with weight.

Figure 18. Blood Hg concentrations versus weight in juvenile Common Loons from Aziscohos and Flagstaff Lakes, 1994-00.



In 1999, we also recaptured four juvenile loons of known age (Table 5). Although sample size precludes conclusions, young loons do appear to have increasing blood Hg levels while maturing on their natal lakes. The rate of Hg increase in these four loons was 1.0 to 3.6% per day.

Table 5. Change in blood Hg concentrations of known-age juvenile loons recaptured 14-40 days later in Maine and New Hampshire, 1999.

Lake	Hg Risk	Change in Days	Change in Hg	Change in Weight	% Increase of Weight	% Increase of Hg	% Increase of Hg/day
Aziscohos	XH	40	0.421	2190	95%	143.2%	3.58%
Aziscohos	XH	22	0.073	1100	51%	20.3%	1.00%
Aziscohos	M	18	0.048	980	52%	32.6%	1.8%
Winnipesauke	M	14	0.101	760	38%	45.5%	3.3%

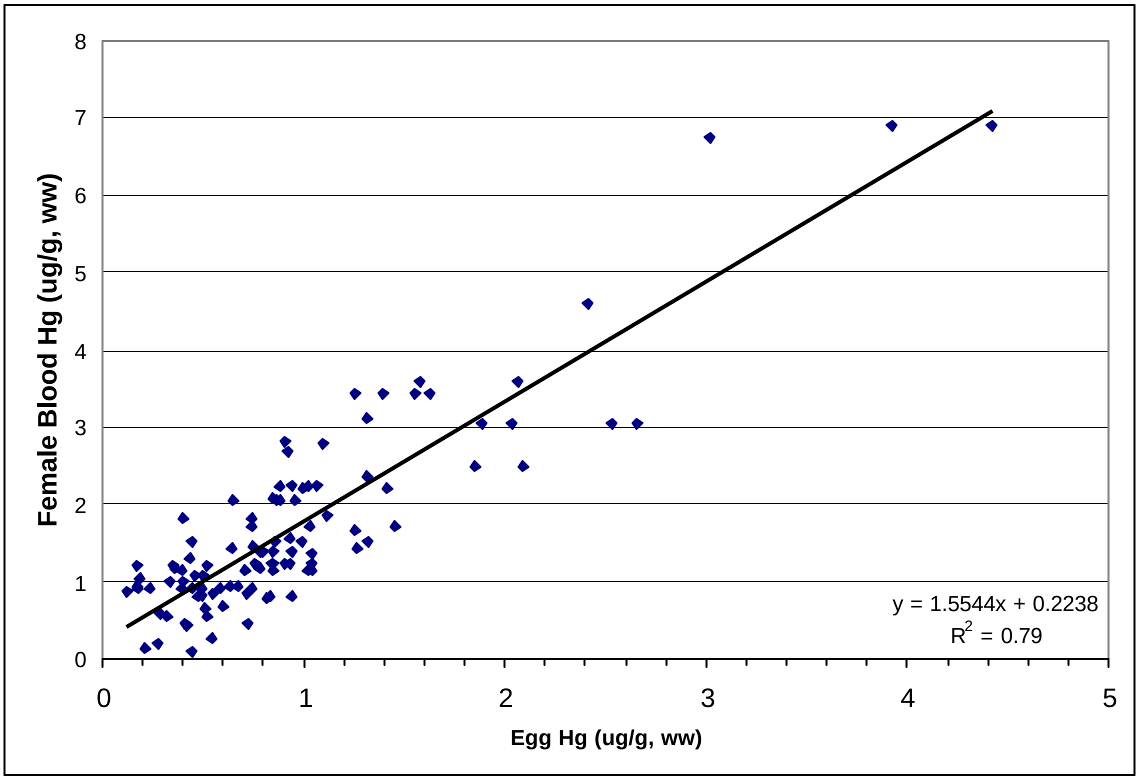
4. Reproduction Relationship with Mercury

a. Egg Development and Hatching Success

Controlled studies have shown that mercury toxicity impacts egg development and hatchability at levels (i.e., 0.5-4.4 ppm) that are found in this study (Borg et al. 1969, Fimreite 1971, Heinz 1979, Spann et al. 1972, Gilbertson 1974). Lower reproductive success in birds has been documented at Hg levels lower than those that cause observable effects on adult behavior and survival (Scheuhammer 1991). Thompson (1996) summarized several controlled studies of captive birds and predicted dietary concentrations of 0.6 ppm (wet weight) (converted from 3.0 ppm dry weight) cause impaired reproduction in birds, yet have little effect on adult survival. Barr (1986) found loons laid fewer eggs when prey Hg averaged 0.3-0.4 ppm and no eggs were laid when prey averaged over 0.4 ppm of Hg.

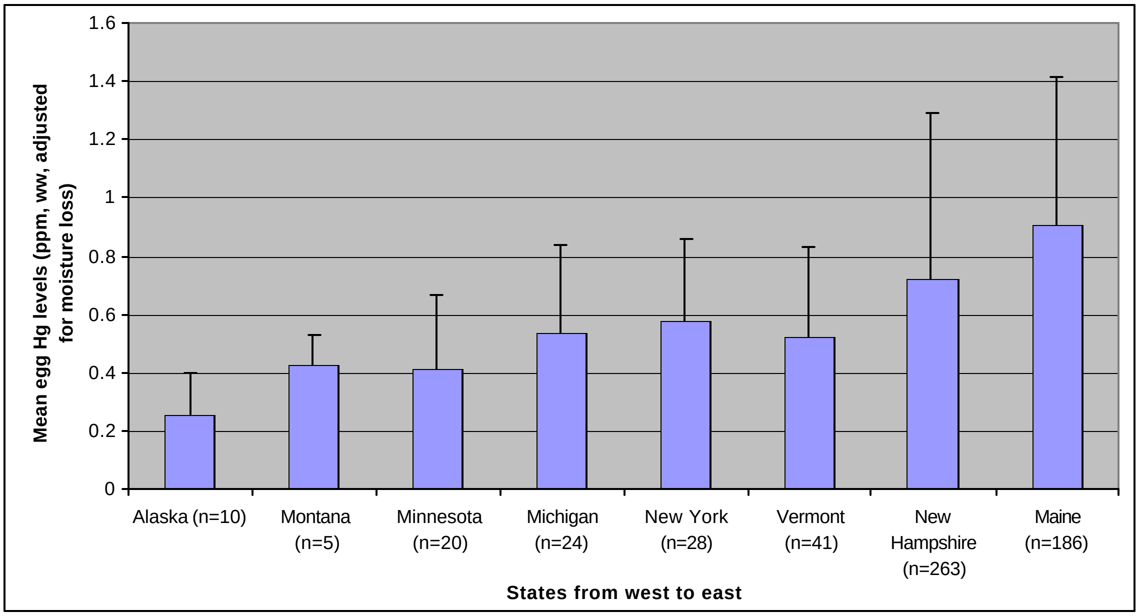
We collected morphometric and developmental information from 199 eggs that were abandoned on Maine lakes because of flooding, human disturbance, and other reasons. Loons typically lay two eggs within 24 hours of one another. Mean egg size in Maine was 145.9 +/-22.1 g (weight), 91.9 +/-3.8 mm (length), 56.9 +/- 2.4 mm (width), and 140.6 +/- 19.8 g (volume). In birds, first-laid eggs are larger than ones following.

Figure 19. Relationship between the Hg levels measured in female blood and their eggs.



Loon egg Hg levels were related to blood Hg levels of associated females (Figure 19). Eggs thereby provide a relevant indication of dietary Hg uptake on breeding lakes. Impacts from elevated Hg levels were detected in egg mass. Evers et al. (In Press) found a significant decline in egg mass as egg Hg levels increased ($p<0.05$). Maine loons had egg Hg concentrations with a mean of 0.91 +/- 0.50 and ranged from 0.12 to 2.65 ppm (ww). Except New Hampshire, mean egg Hg concentrations were significantly higher ($p<0.05$) than other states Alaska, Montana, Minnesota, Michigan, New York, and Vermont (Figure 20) and Canadian provinces (Scheuhammer et al. 2001). New Hampshire egg Hg levels were closest to those found in Maine.

Figure 20. Mean Hg concentrations in Common Loon eggs from selected sites, 1993-01.



b. Impacts on Overall Productivity

Barr (1986) found a strong negative correlation between the successful use of a territory by breeding pairs and Hg contamination (measured in forage fish). Loon reproduction, capacity to lay eggs and maintain nest/territory fidelity, was impacted with fish Hg levels of 0.3 ppm and no reproduction occurred in areas with fish Hg levels of 0.4 ppm. Burgess et al. (1998) also found a significant negative correlation between loon blood Hg levels and reproductive success by using the ratio of nesting vs. territorial pairs (i.e., detects whether an egg was laid). Weaker but negative correlations were also found with hatched young per nesting pair and fledged young per territorial pair. Like the high-risk Hg sites in Kejimkujik NP (Burgess et al. 1998), we found a significant correlation between some of the reproductive success ratios and adult blood, juvenile blood, and egg Hg concentrations ($p<0.05$). Variation and the lack of agreement between the various reproductive success ratios was likely related to confounding variables including predation, density-dependent pressures, human disturbance, storm events, and other mercury-independent factors. Burgess et al. (1998) was able to statistically account for these variables and showed a significant inverse relationship with increasing adult blood Hg levels and decreasing reproductive success.

Egg Laying Success: We found similar inverse relationships with increasing adult blood Hg levels and decreasing reproductive success based on 223 loon territories representing 748 territory-years surveyed (Table 1). The reproductive measure for determining egg-laying success (nesting pair/territorial pair) was similar for low, moderate, and high-risk territories but did exhibit a significant decline ($p<0.05$) of 13% for the territories at highest risk to Hg (Table 6). Small fish Hg levels on many of the extra high-risk lakes had levels that exceeded the 0.30 ppm concentrations that Barr (1986) related to population level impacts.

Egg Hatching Success: How successful the pairs are in hatching their eggs are measured by the reproductive measures, hatching per territorial and hatching per nesting pair. Both of these measures exhibited significant step-down declines in hatching success from low to extra-high risk territories ($p<0.05$) (Table 6). Territorial pairs with extra-high blood Hg levels hatched 30% fewer young than pairs with low Hg risk (<1 ppm). Success by nesting pairs followed a similar, but less exaggerated pattern with a reduction of 19% hatched young from low to extra high risk categories (Table 6).

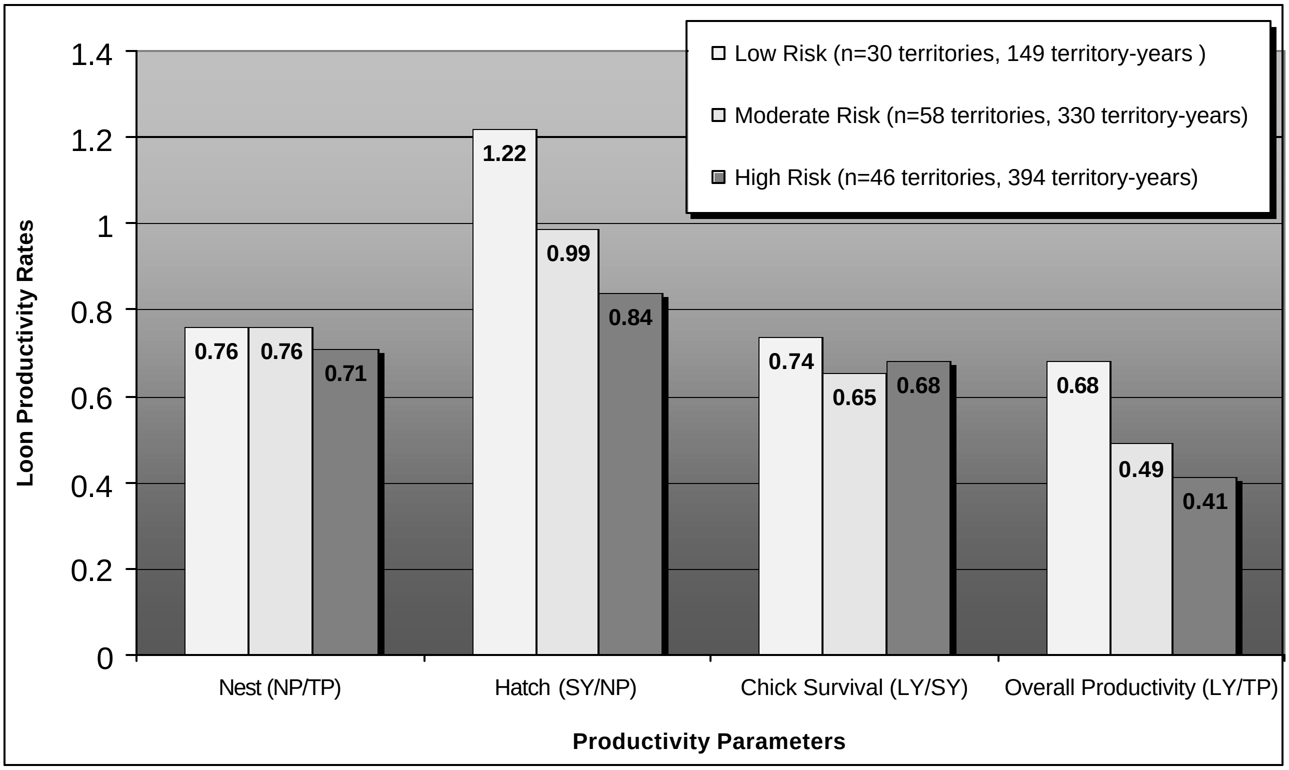
Table 6. Reproductive success of Common Loons in the Rangeley Lakes Region, Maine and selected lakes in New Hampshire.

Level of risk by territory	Low Risk	Mod Risk	High Risk	X-high Risk	Percent change between <NOAEL and >LOAEL
Sample size: # of territories	30	48	22	25	
Sample size: # of territory-years	149	330	173	221	
Nesting Pair/Territorial Pair Ratio	76%	76%	75%	68%	7%
Hatching/Territorial Pair Ratio	0.93	0.75	0.56	0.63	35%
Hatching/Nesting Pair Ratio	1.22	0.99	0.75	0.93	31%
Fledging/Territorial Pair Ratio	0.68	0.49	0.38	0.43	40%
Fledging/Nesting Pair Ratio	0.90	0.65	0.51	0.63	37%
Fledged/Hatched Young Ratio	74%	65%	68%	68%	8%

Chick survival success: Although young loons are rarely confirmed as having actually fledged from their natal lake, the mortality of greater than 6-week-old-young is minimal. Therefore, most monitoring efforts use “large young” as analogous to rates of fledging. We have also used these protocols and found that the survival of young showed a tendency to decline with increasing Hg levels (i.e., 11% decrease in chick survival from low risk to extra high risk). However, because Hg levels in young loons increase as they mature (Figure 18, Table 5) they may be at greatest risk to Hg toxicity just before fledging. Young loons completely molt their flight feathers in by 11 weeks (Barr 1996) and after that have reduced pathways available to depurate the increasing amount of ingested Hg. Therefore, the period between complete remigial molt and actual fledging from the natal lake may be the time of greatest risk.

Overall reproductive success: We also compared fledging rates with territorial and nesting pairs and found patterns similar to hatching ratios (Figure 21). Low risk territorial pairs were 37% more successful in fledging young than high and extra high-risk territorial pairs (Table 6). The fledging/nesting pair ratio exhibited a similar trend. The number of fledged young per territorial pair is one of the more comparable numbers with other monitoring programs.

Figure 21. Summary of change in reproductive measures between three Hg risk categories*.



* Territorial Pair = TP, Nesting Pair = NP, Small Young = SY, Large Young (fledged) = LY

The implications of long-term declines in these reproductive measures are important to address before impacts are perceived in the loss of territorial pairs. New England breeding loon populations (before nesting is initiated) are typically comprised of 54% nesting individuals, 26% territorial but non-nesting individuals, and 20% of non-territorial individuals that are searching for opportunities to attain territorial status (Taylor and Vogel 2000). The number of non-territorial individuals represents the "buffer" in the breeding population and these are the individuals that immediately fill gaps in established territories. A noticeable decline in the number of loon territories that would be detected by traditional surveys would not happen until this "buffer" population was exhausted. If a breeding loon population reached a point where territorial pairs started to disappear, the occasional catastrophic events on the wintering area could have a long-term and substantial impact. Because the average first-year breeding age is 7 years (Evers et al. 2000) and loons typically only fledge 0.52 young per territorial pair (Taylor and Vogel 2000) recovery would be slow. Monitoring marked individuals and modeling their demographics should be initiated to avoid crisis scenarios.

C. Risk Characterization

BRI has measured body burdens of Hg levels in Maine loons and their prey since 1994 in collaboration with members of the Northeast Loon Study Working Group. Cooperators include the U.S. Fish and Wildlife Service, U.S. Environmental Protection Agency, Maine Department of Environmental Protection, Loon Preservation Committee, Maine Audubon Society, Tufts University, and FPL Energy Maine Hydro. Because of these extensive and intensive efforts we were able to access large databases on Hg exposure in fish and loon egg, blood, and feather matrices that are relevant to this report's hazard assessment.

1. Basis for current established risk categories

Samples collected from lakes in other New England states, the Great Lakes region, and the Canadian Maritimes, were used in regional comparisons and for measuring some Hg effects endpoints. We categorized loon territories on single and multi-territorial lakes according to known exposure to MeHg (indicated by blood or eggs). The four risk categories were based on literature and *in situ* studies by the authors and their collaborators (Table 7). Low risk indicates background Hg levels that are minimally impacted by anthropogenic inputs. Loon territories that are in the moderate risk category have elevated MeHg levels but their impact levels on individuals is unknown. Loons that are in the high-risk category are exposed to toxic levels of environmental Hg that potentially have molecular, organism, and/or population effects. The extra high Hg category is based on known impacts on loons and other birds.

Table 7. Risk categories for MeHg (ppm) availability in the Common Loon.

Matrix	Type	Low	Moderate	High	X High	Reference Base
Egg	ww	0-0.5	0.5-1.3	1.3-2.0	>2.0	Evers et al. In Press
Blood-Adult	ww	0-1.0	1.0-3.0	3.0-4.0	>4.0	BRI[1], inferred by Barr 1986 [2]
Blood-Juv.	ww	0-0.1	0.1-0.3	0.3-0.4	>0.4	Meyer et al. 1998[3]
Feather	fw	0-9	9-20	20-35	>35	Thompson 1996, BRI[1]
Prey Fish	ww	0-0.1	0.1-0.3	0.3-0.4	>0.4	Barr 1986, Evers and Reaman 1998

[1] BRI refers to unpublished data by BioDiversity Research Institute

[2] Adult blood Hg levels are generally 10x higher than prey Hg levels (Evers and Reaman 1998) and Barr 1986 found lower reproduction of loons with prey Hg levels of 0.3 ppm and no reproduction at 0.4 ppm.

[3] Applies to 3-5 week-old juveniles, only.

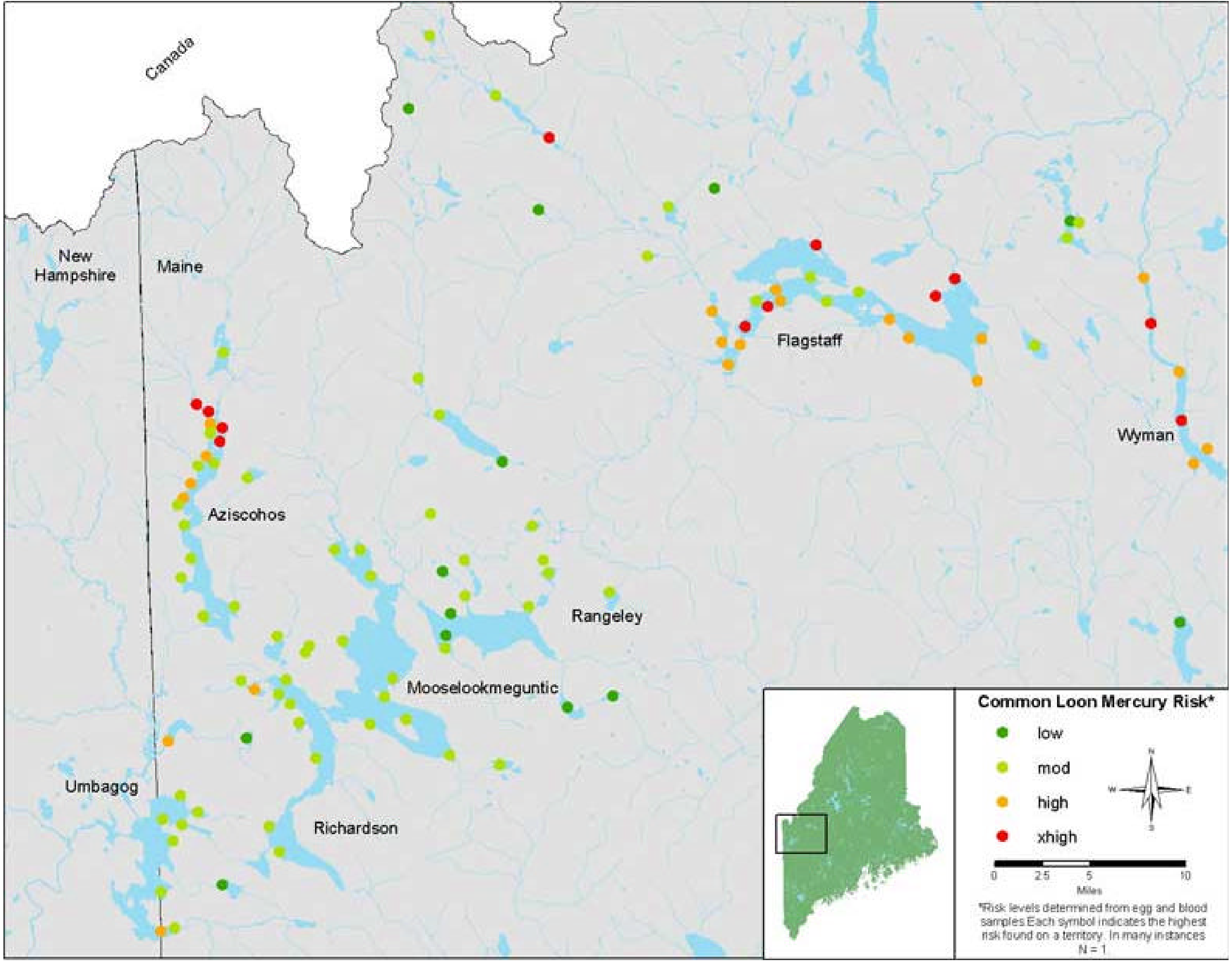
The following exposure assessment follows our risk categories. We have high confidence in adult blood categories based on our findings with corticosterone levels and their step-wise and significant relationship with blood Hg levels (Figure 10). Adult and juvenile blood Hg categories also agreed with associated prey fish Hg levels and compared well to levels found in areas used by Barr (1986) and Meyer et al. (1998). Feather Hg categories are based on extensive literature reviews summarized by Thompson (1996). The egg Hg categories are based on literature reviews for bird egg Hg levels (Scheuhammer et al. 2002) and recent findings by Evers et al. (In Press). The LOAEL for eggs has been changed from 1.0 ppm to 1.3 ppm.

While this "bright line" or point estimate approach to risk does not integrate the actual mechanisms of how Hg impacts wildlife, it does provide an initial, albeit conservative, approach for understanding the extent of risk. At present, the upper limit of the low risk category is considered the no observed adverse effect level (NOAEL) and the lower limit of the high risk category is the lowest observed adverse effect level (LOAEL). Future analysis will incorporate more statistically robust approaches that investigate the probability of the risk to the target population using dose-response curves (i.e., Rumbold et al. 1999, Sample and Suter 1999).

2. Opportunistic sampling in the Rangeley Lakes Region

Opportunistic sampling efforts in the Rangeley Lakes Region indicate four areas of high Hg risk as measured in loons and fish (Figure 22). They include northern Aziscohos Lake, southern Chain-of-Ponds, most of Flagstaff Lake, and Wyman Lake. Each of these water bodies have water level fluctuations that exceed 1.5 m during the summer (June through August). Evers and Reaman (1998) have shown a significant relationship between biotic Hg levels and summer water changes >1.5m. Several reservoirs have relatively low levels of biotic Hg, including Mooselookmeguntic, Rangeley, Richardson, and Umbagog. Most natural lakes also exhibit low Hg risk to their biota. Exceptions include Big Beaver Pond and Sturtevant Lake.

Figure 22. Risk characterization based on opportunistic sampling efforts in the Rangeley Lakes Region.



3. Statewide Random Sampling

In recognition of widespread environmental contaminants, the USEPA uses the Environmental Monitoring and Assessment Program (EMAP) as a long-term tool for monitoring and assessing ecological condition (e.g., effectiveness of the Clean Air Act). The monitoring of surface waters using EMAP's probability-based surveys for ecological indicators provides a statistically valid technique for making regional and eventually national extrapolations of the exposure and effects of various environmental stressors (e.g., Whittier et al. 1997, Yeardley et al. 1998). The complementary regional program, REMAP, has also proven effective in this same regard for Maine (e.g., Stafford and Haines 1997, Mower et al. 1997).

We surveyed 54 lakes in 2001 and found 71 loon territorial pairs that produced 33 large chicks (i.e., >6 weeks) on 35 lakes (Table 8, Figure 23). Because our loon sampling efforts during the breeding season are limited to adults with chicks we were able to collect loon blood samples from 17 lakes (an egg represented one lake). Characterizing risk for this and past sampling efforts indicates Hg risks 19% of Maine’s breeding loon population.

Table 8. Common Loon sampling summary on REMAP waterbodies (all years 1997-2001).

Midas #	Lake	Most recent year of sampling	Sample types w/ known Hg[1]	# loon territories w/ Hg	Low Hg Risk	Mod Hg Risk	High Hg Risk	Xhigh Hg Risk
3898	Balch	1998	B,E	1	0	1	0	0
4702	Bottle	2001	B	1	0	0	0	1
2516	Canada Falls	2001	B	1	0	0	0	1
5064	Chain of Ponds	2001	B,E	2	0	1	0	1
2752	Chase	2001	E	1	0	1	0	0
2856	Churchill	2001	B	2	0	2	0	0
5236	Cobbosseecontee	2001	B,E	1	0	1	0	0
5400	Damariscotta	1998	B	1	0	1	0	0
5349	East	1998	B	1	0	1	0	0
0078	Embden	2001	B	1	0	1	0	0
4282	Field’s	2001	B	1	0	1	0	0
4322	Jacob Buck	1999	B	1	0	0	0	1
3272	Keewaydin	2001	B	1	0	1	0	0
5024	Little Ossipee	1997	B	1	0	0	0	1
5250	Little Purgatory	2001	B	1	0	1	0	0
2536	Long	1997	B	1	0	1	0	0
3760	Lower Range	1999	B	1	0	1	0	0
5496	Passagassawaukeag	2001	B	1	0	1	0	0
5198	Pease	2001	B,E	1	0	1	0	0
1100	Pleasant	2001	B	1	1	0	0	0
4330	Rocky	2001	B	1	0	1	0	0
3566	Sandy River	2000	B	1	1	0	0	0
1134	Second	2001	B	1	0	1	0	0
9931	Togus	2001	B	1	0	1	0	0
3102	Umbagog	2001	B,E	9	0	9	0	0
2630	Wood (Little Big)	2001	B	1	0	1	0	0

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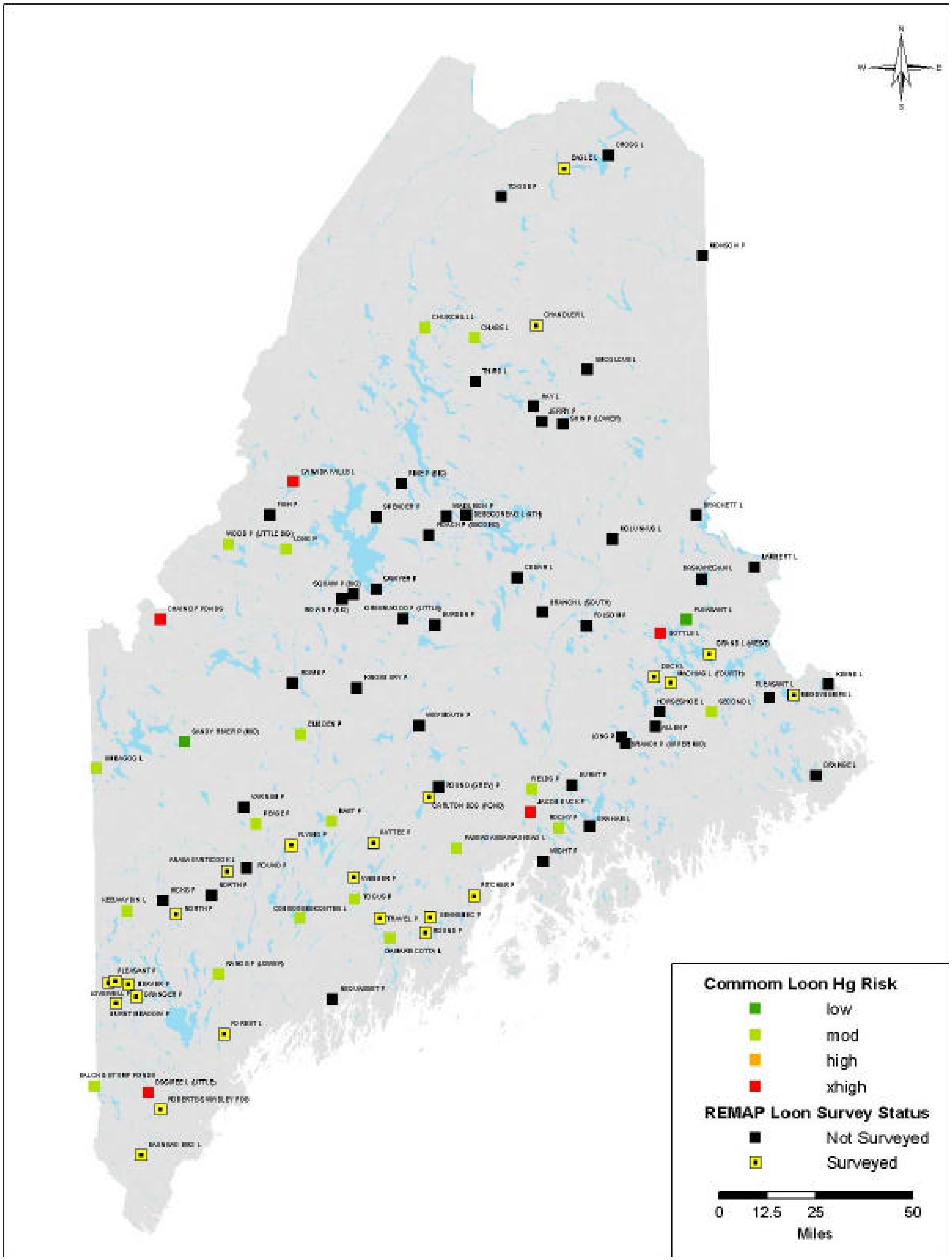
[1] In Lakes/territories where Hg is known for both egg and whole blood, the Hg risk value represents the blood level (B = Whole blood; E = Egg).

Total	**Territories**	**28 in 2001**	**-**	**36**	**2**	**29**	**0**	**5**
Total	**Lakes**	**18 in 2001**	**-**	**26**	**2**	**19**	**0**	**5**
Risk	**% Territories at risk**	**(n=36)**	**-**	**-**	**6%**	**80%**	**0%**	**14%**
Risk	**% Lakes at risk**	**(n=26)**	**-**	**-**	**8%**	**73%**	**0%**	**19%**

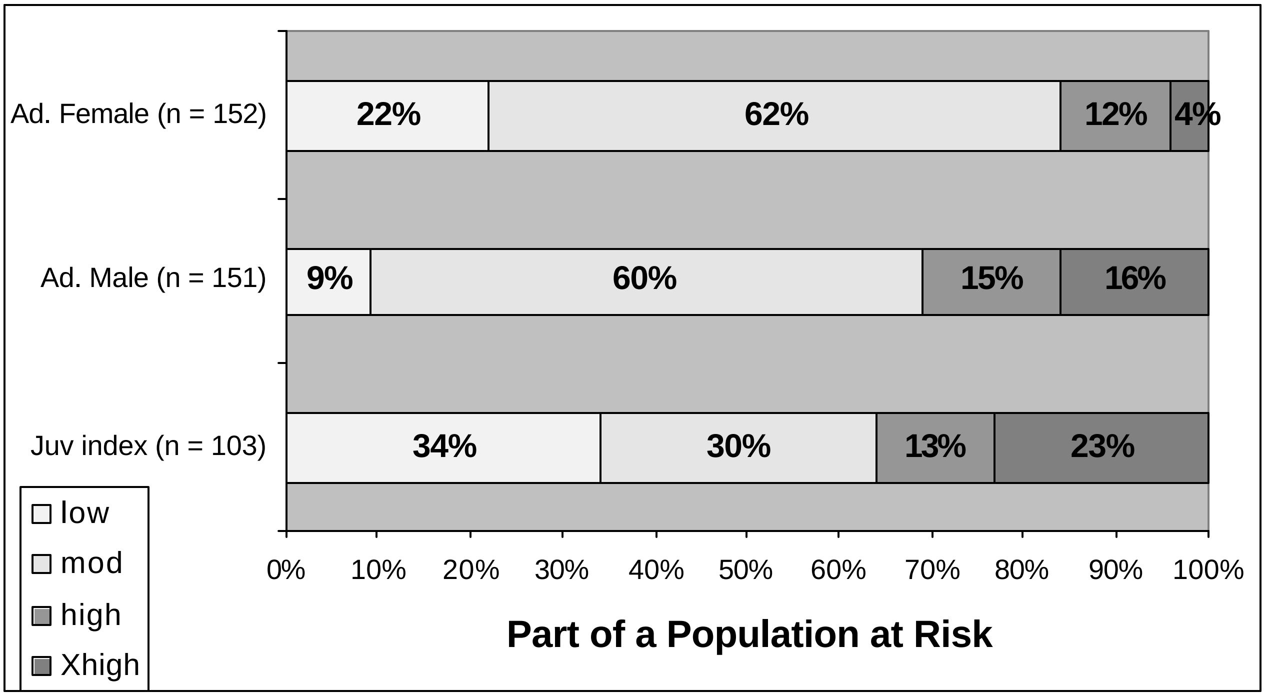
Figure 23. Distribution of Hg risk according to REMAP lakes for Maine.


N
W
E
S
CHANDLER L
JERRY P
BRACKETT L
LAMBERT L
PLEASANT L
BOTTLE L
HORSESHOE L
SECOND L
ALLEN P
LONG P
FISH P
LONG P
SPENCER P
SAWYER P
SQUAW P (BIG)
CEDAR L
BRANCH L (SOUTH)
CHAIN OF PONDS
SANDY RIVER P (BIG)
UMBAGOG L
FIELDS P
JACOB BUCK P
ROCKY P
CARLTON BOG (POND)
FLYING P
NORTH P
TOGUS P
TRAVEL P
DAMARISCOTTA L
PLEASANT P
FOREST L
ROBERTS-WADLEY POND
Commom Loon Hg Risk
low
mod
high
xhigh
REMAP Loon Survey Status
Not Surveyed
Surveyed
0
12.5
25
50
Miles

4. Common Loon mercury risk profiles

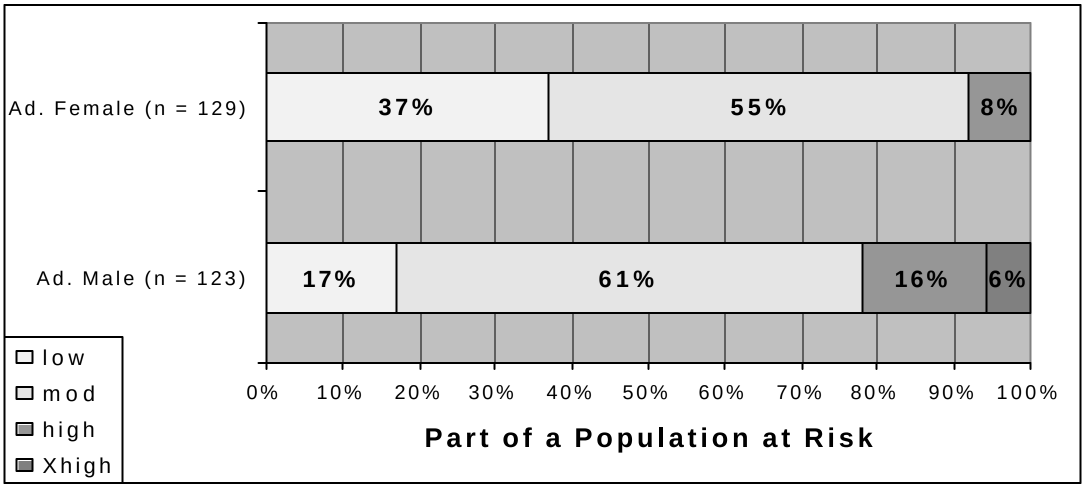
Adult male loons (36%) have greater risks to MeHg availability in their prey than female loons (19%) (Figure 24). Other geographic areas outside Maine also have significant differences between gender Hg levels (Evers et al. 1998a). Individual Maine male loons are at greater risk than females because of their tendency to eat larger fish with higher levels of Hg and the female's ability to depurate some of her Hg body burden through eggs. Because we capture juvenile loons at different ages and we know they increase in Hg levels through the summer we indexed juvenile Hg levels by weight (as an indicator of age). A total of 34% of Maine's juvenile loon population is at risk.

Figure 24. Exposure profile for adult and juvenile loon blood in four Hg risk categories in Maine, 1994-01.



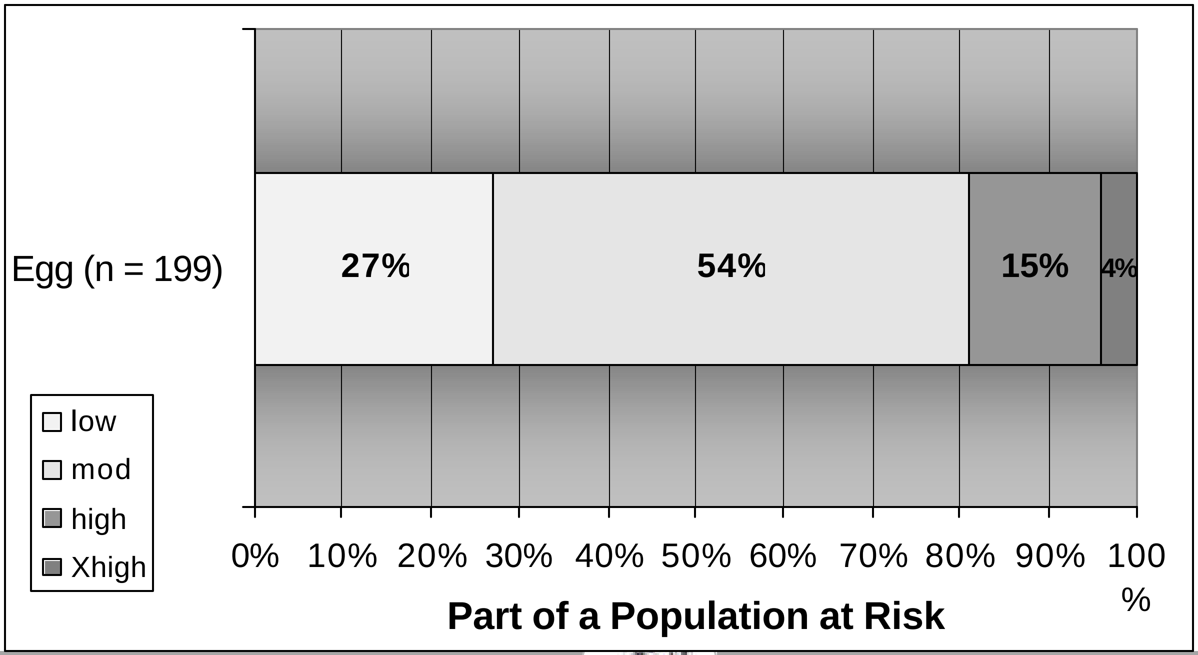
Feathers provide insight into risk to individuals and incorporate age dependent factors. There is a significant gender difference ($p<0.05$) in Maine as well as across the country. Upwards of 22% of Maine's male loons have Hg body burdens at levels that place them at high risk to Hg toxicity (Figure 25). A total of 8% of the females are at risk. The significantly increasing annual rate of Hg uptake into the body burden (9% for males and 5.6% for females) indicates that high risk individuals will likely be replaced in the breeding population by younger individuals with a lower body burden of Hg. This Hg-induced replacement rate could conceivably impact individual performance and therefore the integrity of the breeding population.

Figure 25. Exposure profile in adult male and female loon feathers in four Hg risk categories in Maine, 1994-01.



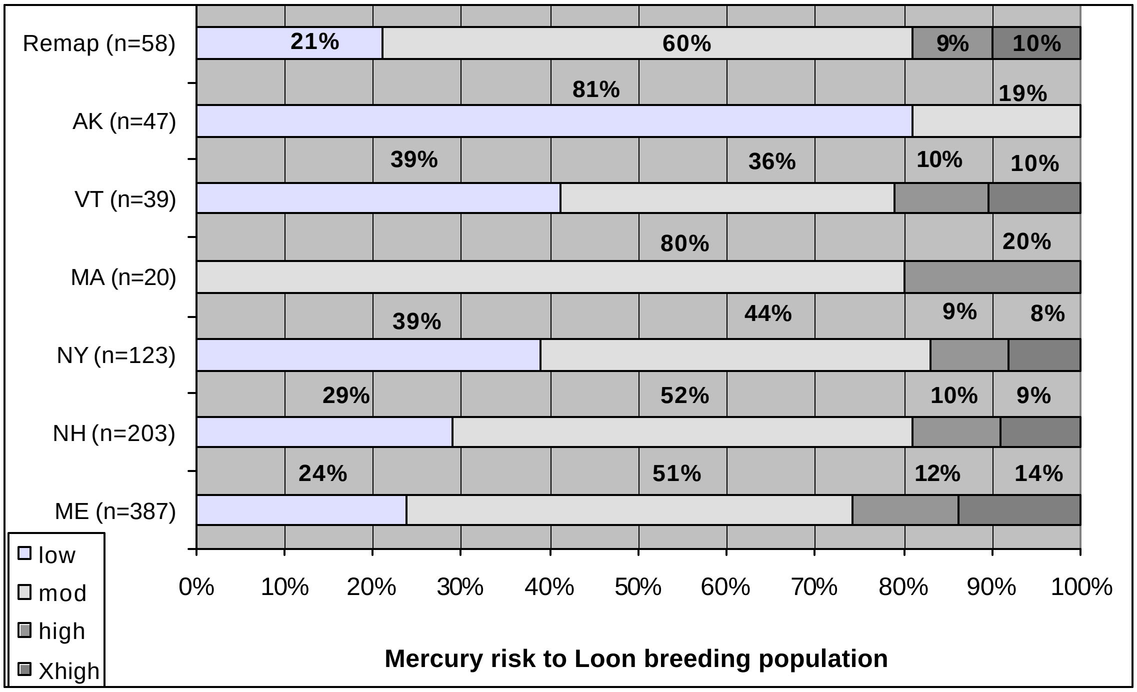
According to risk levels set by several studies on loons and other species, 46% of the eggs laid by Maine loons are at high risk to Hg toxicity (Figure 26). Although some species of birds can apparently tolerate much higher levels of Hg in the eggs without impact (e.g., Herring Gull), the loon is likely far more sensitive to Hg levels in its eggs. The extra high risk category of greater than two ppm is widely accepted as showing high mortality in many bird species (Thompson 1996). Our high-risk category is based on Barr (1986) because eggs were not hatching at his study site when they exceeded one ppm. Further studies may find that loons are able to tolerate higher egg Hg levels and we could then refine the high-risk category. Until then, this category is acceptable particularly when other birds are impacted at egg Hg levels of 0.5 ppm (Thompson 1996).

Figure 26. Exposure profile for Maine loon eggs in four Hg risk categories, 1994-01.



The cumulative risk level of Hg for loons is based on the premise that individual impacts of Hg can be determined through blood and egg measurements (Figure 27). The combination of these two matrices provides a comprehensive picture of Hg exposure that can be related to the breeding lakes. The few eggs laid by females that had blood drawn were not included here to avoid statistical problems of repeated measures. Egg Hg levels correlate with female blood Hg levels and are therefore representative measures of the MeHg bioavailability on the breeding lake. Repetitive measures of blood Hg levels for individuals were averaged to provide one value. Feather Hg levels were not included in the cumulative risk assessment.

Figure 27. Distribution of the cumulative risks of Hg impacts for three matrices (blood, feather, and egg) in New England including comparisons with Alaska (our reference site) and REMAP lakes, 1994-01.

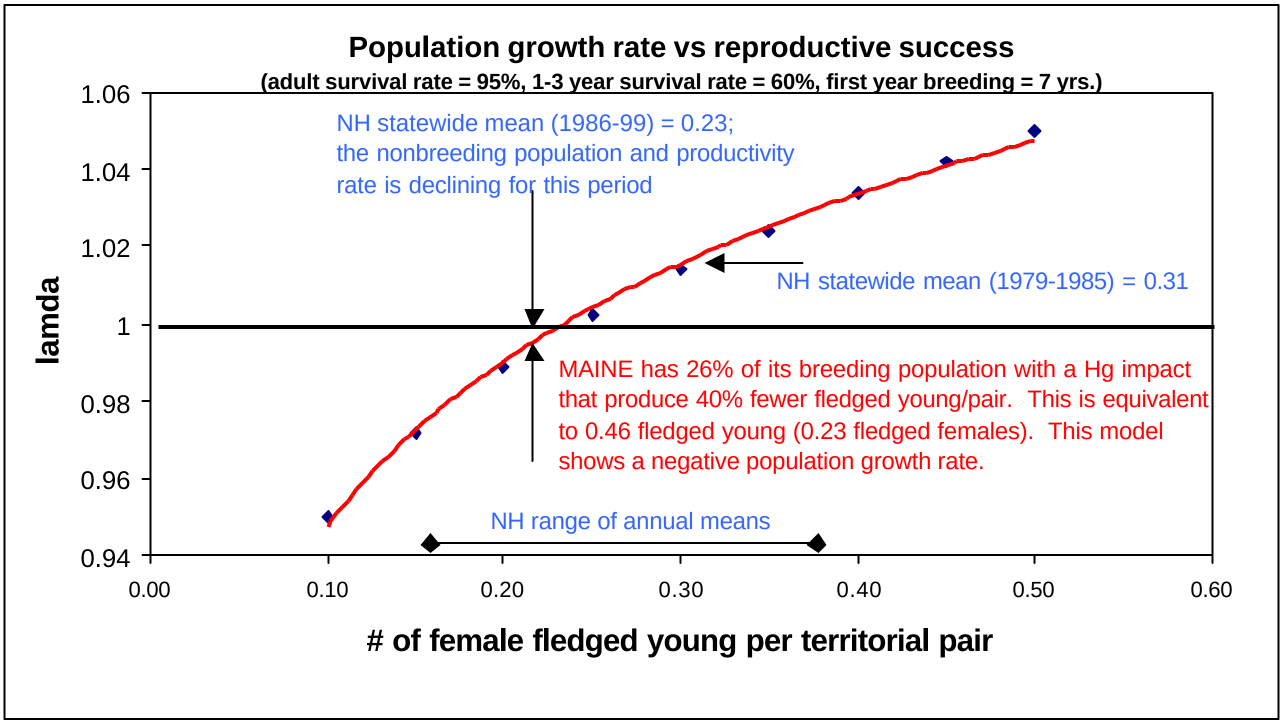


Based on opportunistic sampling strategies, current levels of Hg in Maine cause 26% of the breeding population to be at risk to behavioral, physiological, survival, or reproductive changes that are not congruent with healthy loon populations. Compared to other New England states, the risk to Maine's loons is higher. New Hampshire and New York also have "hotspots" of MeHg bioavailability that are likely related to negative population growth rates.

Although 26% of Maine's loons are at risk to impacts from Hg and these risks create a "bottom line" decline in their reproductive success of 40%, the overall impact at the population level is needed. A model was developed in collaboration with the USEPA-Office of Research and Development in Narragansett, Rhode Island to determine how the risk of Hg impacts Maine's breeding loon population (Figure 28). This model is based on the collection of three demographic parameters collected across New England and the Great Lakes (Evers et al. 2000, Evers 2001). Annual adult survival for breeding loons is 95%. The annual survival of loons at age one through three is 60%. And, the average age for the first year of breeding is 7 years. Using stage-classified matrix models a deterministic model (Caswell 2001) was developed to better understand the impacts of stressors such as Hg on the loon's breeding population.

We found that current Hg impacts are creating a negative population growth rate for Maine's Common Loon breeding population. Because our modeled results hover near lamda, refinement of this model is a high priority. Comparison of New Hampshire's loon-Hg relationship indicates similar population growth rate tendencies. Unlike Maine's loon population, monitoring efforts in New Hampshire cover the entire population and 59% of the territories have known Hg risk levels. Because it appears that Maine's loon population is at higher risk than New Hampshire's, the Maine negative population growth rate is likely a conservative estimate. Another indication of mercury's potential impact on Maine's loon population is the significant decline in New Hampshire's loon productivity from the period 1979-1985 (0.31 fledged female young) to the period 1986-1999 (0.23 fledged female young).

Figure 28. A model of the Common Loon's population growth rates based on parameters from loons across New England the Great Lakes. Comparison of the current impacts of Hg on loons indicates a negative population growth rate.



D. Wildlife Criterion Value

Our approach to a high resolution risk characterization for the Common Loon provides the necessary information for developing a Maine-based WCV. Recent efforts by the USEPA have established a generic WCV with several major limitations that we are improving with this study. The WCV estimates wildlife population viability through measurement of contaminant stressors such as surface water Hg concentrations (Nichols and Bradbury 1999).

1. Tested Dose

The reference dose is the tested dose of Hg that causes impacts divided by the product of applicable uncertainty factors. Until we have more information on our uncertainty factors we are using the avian wildlife tested dose of 78 ug/kg bw/day.

2. Uncertainty factors

Uncertainty factors identified by Nichols and Bradbury (1999) were not quantified in 2001and we therefore default to using their values: Uncertainty factor for species-to-species (UF_A) equals 3, uncertainty factor for subchronic to chronic (UF_S) equals 1) and uncertainty factor from LOAEL to NOAEL (UF_L) equals 2).

3. Loon weights

Field capture of healthy, breeding loons in Maine shows mean female weight of 4,714 +/- 302g (n=157, range=4,000 to 4,800) and mean male weight of 5,953 +/-412g (n=153, range=4,600 to 7,550). Combining sexes, the mean breeding loon weight in Maine is 5,326 +/- 717 (n=310).

4. Diet fraction and amount

To determine prey Hg concentrations in species and size categories that loons select, we developed a sampling protocol based on Barr (1996). He found, adult loons from central Ontario consumed 432g (small fish), 365g (medium-sized fish), and 163g (large fish) daily or approximately 20% of their body weight per day. The percentage of individual fish consumed by size class was: 80% small, 18% medium, and 2% large. Barr (1996) determined that because of their "zig zag" swimming behavior, yellow perch (*Perca flavescens*) were the preferred food of adult and juvenile loons. Median size perch consumed during Barr's trials for 12-15 week old loons (adult sized individuals) measured 5.0 cm and ranged from 1.0-9.5cm. Adult loons commonly ate perch up to 12.0-16.0 cm. Barr (1986) found the lowest observed adverse effect level (LOAEL) for adult loons feeding on fish with whole body Hg concentrations (wet weight) over 0.30 ppm (i.e., impaired reproduction) and 0.40 ppm (i.e., no reproduction).

Male and female loons from Maine and New Hampshire are 26% larger than their counterparts in western Ontario. Therefore, we adjusted the amount of fish consumed by the heavier New England loons for small, medium, and large/extra large size class: 45%, 38% and 17% for females and 33%, 40% and 27% for males. Therefore, we estimated respective trophic level 3 and 4 fractions for each size class as 83% and 17% for females and 73% and 27% for males. Loons eat approximately 20% of their body weight per day.

5. Bioaccumulation factor

First-year measurements of exposure parameters indicate a BAF of 75,000 for trophic level 3 and 120,000 for trophic level 4 based on the relationship of total Hg in unfiltered water with total Hg in yellow perch.

6. WCV for the Common Loon

Using the algorithm and reference dose from Nichols and Bradbury (1999) and our exposure parameters (Table 9) we calculated the WCV for male loons as 746 pg Hg/L and for female loons as 786 pg Hg/L. The Great Lakes Water Quality Initiative (GLI) uses an avian species WCV of 1,300 pg Hg/L. Without incorporating a reference dose based on population level impacts to loons reduces the precision of our Maine-based WCV, however, when using the GLI-accepted reference dose we found a Maine-based WCV that is more protective.

$$\mathrm{WCV}_{\text{Male Loon}} = \frac{0.078\ \text{mg/kg/d} \times [1/3 \times 1 \times 2)]) \times 5.95\ \text{kg}}{0.012\ \text{L/d} + [(0.73)\,(1.19\ \text{kg/d} \times 75{,}000) + (0.27)\,(1.19\ \text{kg/d} \times 120{,}000)}$$

$$\mathrm{WCV}_{\text{Male Loon}} = 746\ \text{pg/L}$$

$$\mathrm{WCV}_{\text{Female Loon}} = \frac{0.078\ \text{mg/kg/d} \times [1/3 \times 1 \times 2)]) \times 4.71\ \text{kg}}{0.012\ \text{L/d} + [(0.83)\,(0.943\ \text{kg/d} \times 75{,}000) + (0.17)\,(0.943\ \text{kg/d} \times 120{,}000)}$$

$$\mathrm{WCV}_{\text{Female Loon}} = 786\ \text{pg/L}$$

Table 9. Exposure parameters and test dose.

	Body Weight, WT_A (g)	Ingestion Rate, F_A (g/day)	Percent of diet at each trophic level*	Test Dose
Loon – Male	5,953 +/- 412	1,190 +/- 82	Trophic 3=73%, 4=27%	78 ug/kg bw/day
Loon - Female	4,714 +/- 302	943 +/- 60	Trophic 3=83%, 4=17%	78 ug/kg bw/day

* Entire diet is from aquatic source

RECOMMENDATIONS

The weight of evidence strongly suggests impact of Hg on a significant component of the breeding loon population and likely other piscivorous wildlife in Maine. This information base begins to address two legislative strategies. First, in the 1997 Annual Report, The Land and Water Resources Council submitted an evaluation and recommendations to the Joint Standing Committee on Natural Resources on 28 January 1998 (LWRC 1998). In strategy 9, in regard to focusing on "biological research on the effects of mercury on the health of loons, fish, and other wildlife with elevated mercury levels," the Council proposed the action to "include projects aimed at documenting any reproductive or other effects which may be associated with elevated mercury levels." This was to be in partnership and funded by the second legislative-directed program—the Surface Water Ambient Toxics program (SWAT). In a 1995 technical report, SWAT (1997) included as their goals that "Maine's lakes, rivers, and streams, and marine and estuarine waters will be monitored for the occurrence of toxic pollutants on an ongoing basis" and that "water, sediment, and biological tissues and aquatic communities will be tested as necessary to indicate exposure to and impacts by toxic contaminants" and "data from the program will be used to support assessment of risks to human and ecological health posed by toxic contaminants."

The results from this study and future work will help enable MDEP to meet these strategy goals by (1) documenting the extent of Hg contamination to the state's lakes and downstream rivers, (2) using as evidence for ecological damage to public resources and for setting appropriate science-based policy, (3) providing regional and national policy makers with science-based reasons for restricting Hg emissions, and (4) serving as a reference for detecting future changes in atmospheric Hg deposition. We recommend further development of the WCV and associated studies using the Common Loon as the primary ecological indicator of aquatic integrity for multiple geographic and ecological scales in Maine. Specific task for 2002 include:

1) Continue the population-level analysis of Hg impacts on various reproductive measures by targeting 30 territories of each risk category to minimize NOAEL to LOAEL uncertainty;
2) Continue a sampling strategy that is probability-based across Maine using the REMAP lake list.
3) Continue improving sample size and precision of the water and fish Hg database to further develop bioaccumulation factors;
4) Integrate measurements of Hg from other species to minimize species-to-species uncertainty;

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Appendix 1

This project is designed to also meet future goals of regional and national interest that include (1) assembling the wealth of existing toxicological and demographic information into an integrated format, (2) filling in data gaps identified through demographic models and evaluating current inadequate sample sizes, (3) improving resolution of spatially explicit toxicological and demographic information through analysis of genetic population structure, and (4) organizing the existing and newly collected databases into a stressor-risk rank matrix that will provide a basis for spatially-explicit models of landscape level impacts from critical environmental stressors such as MeHg availability and marine oil spills (Figure 2).

FIGURE 2. Conceptual framework for bioassessment model of multiple stressors of the Common Loon.